



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification: A61F 2/00, A61K 9/14, A61K 9/50, C08F 8/00, C08F 283/04, C08G 63/02, C08G 63/08, C08G 63/44, C08G 63/48, C08G 63/91, C08G 69/10, C08G 69/44, C08G 69/48, C08L 67/00, C08L 71/02, C08L 77/00	A1	(11) International Publication Number: WO 00/33764 (43) International Publication Date: 15 June 2000 (15.06.2000)
--	-----------	---

(21) International Application Number: PCT/US99/28718 (22) International Filing Date: 03 December 1999 (03.12.1999) (30) Priority Data: 60/110,849 04 December 1998 (04.12.1998) US (60) Parent Application or Grant PATHAK, Chandrashekhar, P. [/]; O. PATHAK, Chandrashekhar, P. [/]; O. SAWHNEY, Amarpreet, S. [/]; O. EDELMAN, Peter, G. [/]; O. PISANO, Nicola, A. ; O.	Published
---	------------------

(54) Title: BIOCOMPATIBLE CROSSLINKED POLYMERS
(54) Titre: POLYMERES RETICULES BIOCOMPATIBLES

(57) Abstract

Biocompatible crosslinked polymers, and methods for their preparation and use, are disclosed in which the biocompatible crosslinked polymers are formed from water soluble precursors having electrophilic and nucleophilic groups capable of reacting and crosslinking in situ. Methods for making the resulting biocompatible crosslinked polymers biodegradable or not are provided, as are methods for controlling the rate of degradation. The crosslinking reactions may be carried out in situ on organs or tissues or outside the body. Applications for such biocompatible crosslinked polymers and their precursors include controlled delivery of drugs, prevention of post-operative adhesions, coating of medical devices such as vascular grafts, wound dressings and surgical sealants.

(57) Abrégé

L'invention concerne des polymères réticulés biocompatibles, ainsi que leurs procédés de préparation et d'utilisation. Ces procédés consistent à former les polymères réticulés biocompatibles à partir de précurseurs, solubles dans l'eau, porteurs de groupes électrophiles et nucléophiles capables d'une réaction et d'une réticulation in situ. L'invention concerne également des procédés permettant de rendre les polymères réticulés biocompatibles obtenus biodégradables ou non, ainsi que des procédés permettant de réguler la vitesse de dégradation. Les réactions de réticulation précitées peuvent être réalisées in situ sur les organes ou les tissus ou à l'extérieur du corps. Parmi les applications relatives à ces polymères réticulés biocompatibles et à leurs précurseurs, on peut citer la libération contrôlée de médicaments, la prévention d'adhérences post-opératoires, le revêtement de dispositifs médicaux tels que les greffes vasculaires, les pansements et les substances chirurgicales d'étanchéité.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



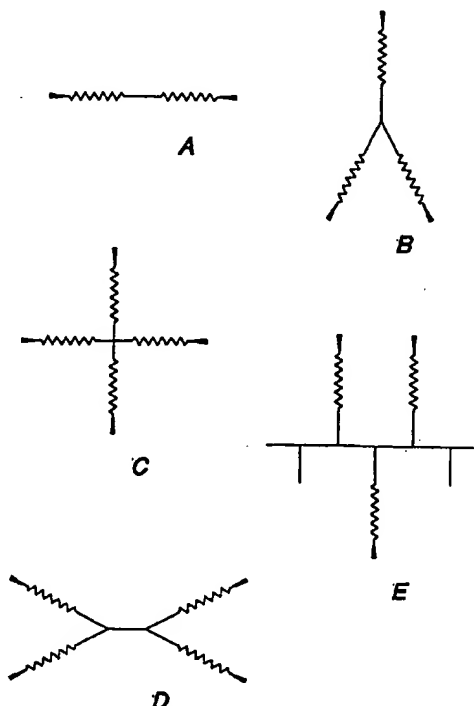
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification 7 : A61F 2/00, A61K 9/14, 9/50, C08F 8/00, 283/04, C08G 63/02, 63/08, 63/44, 63/48, 63/91, 69/10, 69/44, 69/48, C08L 67/00, 71/02, 77/00</p>	<p>A1</p>	<p>(11) International Publication Number: WO 00/33764 (43) International Publication Date: 15 June 2000 (15.06.00)</p>
<p>(21) International Application Number: PCT/US99/28718 (22) International Filing Date: 3 December 1999 (03.12.99) (30) Priority Data: 60/110,849 4 December 1998 (04.12.98) US (71)(72) Applicant and Inventor: PATHAK, Chandrashekhar, P. [US/US]; 16113 Braesgate Drive, Austin, TX 78717 (US). (72) Inventors: SAWHNEY, Amarpreet, S.; 12 Idylwild Road, Lexington, MA 02421 (US). EDELMAN, Peter, G.; 8 Woodstock Circle, Franklin, MA 02038 (US). (74) Agents: PISANO, Nicola, A. et al.; Fish & Neave, 1251 Avenue of the Americas, New York, NY 10020 (US).</p>		<p>(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p>

(54) Title: BIOCOMPATIBLE CROSSLINKED POLYMERS

(57) Abstract

Biocompatible crosslinked polymers, and methods for their preparation and use, are disclosed in which the biocompatible crosslinked polymers are formed from water soluble precursors having electrophilic and nucleophilic groups capable of reacting and crosslinking in situ. Methods for making the resulting biocompatible crosslinked polymers biodegradable or not are provided, as are methods for controlling the rate of degradation. The crosslinking reactions may be carried out in situ on organs or tissues or outside the body. Applications for such biocompatible crosslinked polymers and their precursors include controlled delivery of drugs, prevention of post-operative adhesions, coating of medical devices such as vascular grafts, wound dressings and surgical sealants.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UC	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LJ	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

D scription

5

10

15

20

25

30

35

40

45

50

55

5

10

15

BIOCOMPATIBLE CROSSLINKED POLYMERS

20

Field Of The Invention

The present invention relates generally to biocompatible crosslinked polymers, methods for preparing and using same.

25

Background Of The Invention

In the field of medicine there has been a growing recognition of the benefits of using biocompatible crosslinked polymers for the treatment of local diseases. Local diseases are diseases that are manifested at local sites within the living animal or human body, for example atherosclerosis, postoperative adhesions, rheumatoid arthritis, cancer, and diabetes. Biocompatible crosslinked polymers may be used in drug and surgical treatments of such diseases.

30

35

15

40

Historically, many local diseases have been treated by systemic administration of drugs. In this approach, in order to achieve therapeutic levels of drugs at local disease sites, drugs are delivered (via oral administration or injection) at a high systemic concentration, often with adverse side effects. As an alternative, biocompatible crosslinked polymers may be used as carriers to deliver drugs to local sites within the body, thereby reducing the need for the systemic administration of high concentrations of drugs, while

45

50

55

enhancing effectiveness.

Local diseases also have been treated with surgery. Many of these surgical procedures employ devices within the body. These devices may often be formed from or coated with biocompatible crosslinked polymers. For example, a surgical sealant is a device formed from biocompatible crosslinked polymers that may be used to reduce migration of fluid from or into a tissue. For surgical sealants, as with many other surgical procedures, it is sometimes necessary to leave devices in the body after surgery to provide a continuing therapeutic benefit. In such cases, it may be desired that the implant biodegrade over time, eliminating the need for a second surgical procedure to remove the implant after its usefulness has ended. Regardless of whether the implant biodegrades over time, it may also be used, as described above, to deliver drugs to local sites within the body.

Many surgical procedures are now performed in a minimally invasive fashion that reduces morbidity associated with the procedure. Minimally invasive surgery ("MIS") encompasses laparoscopic, thoracoscopic, arthroscopic, intraluminal endoscopic, endovascular, interventional radiological, catheter-based cardiac (such as balloon angioplasty), and like techniques. These procedures allow mechanical access to the interior of the body with the least possible perturbation of the patient's body. Biocompatible crosslinked polymers may be advantageously used to form or coat many of these MIS tools. These polymers may also be used to form sutures, surgical clips, staples, sealants, tissue coatings, implants and drug delivery systems.

Most of the polymers used with MIS applications are pre-formed to a specific shape before being used in a given application. However, such pre-formed objects have limitations in MIS procedures because they, like other

5 large objects, are difficult to transport through the
small access sites afforded by MIS techniques. In
10 addition, the shape of the pre-formed object may not be
appropriate because the target tissues where such objects
5 are likely to be used have a variety of shapes and sizes.
To overcome these limitations, in situ curable or gelable
biocompatible crosslinked polymer systems have been
15 explored. The precursors of such systems are usually
liquid in nature. These liquids are then transported to
10 the target tissue and applied on the target organ or
tissue. The liquid flows and conforms to the shape of
20 the target organ. The shape of the conformed liquid is
then preserved by polymerization or a gelation reaction.
This approach has several advantages, including
15 conformity to organ shapes and the ability to implant
large quantities of liquid using MIS procedures.

One use of in situ curable biocompatible
crosslinked polymers in MIS procedures is to form tissue
coatings so as to prevent post-surgical adhesions. For
30 example, J.L. Hill-West et al., "Prevention of
Postoperative Adhesions in the Rat by In Situ
Photopolymerization of Bioresorbable Hydrogel Barriers,"
35 Obstetrics and Gynecology, 83(1):59 (1994) describes the
use of free radical photopolymerizable water-soluble
25 monomers to form biocompatible crosslinked polymers and
thereby prevent post-operative adhesions in two animal
40 models. U.S. Patent No. 5,410,016 to Hubbell et al.
describes the use of free radical photopolymerizable
monomers to form biocompatible crosslinked polymers,
30 which then are used as tissue adhesives, controlled-
45 release carriers and as tissue coatings for the
prevention of post-operative adhesions.

Free Radical Polymerization

Many of the biocompatible crosslinked polymers
50 previously known used free radical polymerization of
35 vinylic or acrylic functionalities. For example, the

5 Hill-West article describes the use of free radical
photopolymerizable, water soluble monomers consisting of
10 8000 molecular weight ("MW") polyethylene glycol ("PEG")
extended at both ends with oligomers of lactic acid and
5 further acrylated at both ends. The aforementioned
Hubbell patent describes the use of acetophenone
derivative or eosin initiated free radical polymerization
15 of acrylic functionalities of water-soluble biodegradable
macromolecules. U.S. Patent No. 4,938,763 to Dunn
20 describes the use of benzoyl peroxide initiated free
radical polymerization of liquid prepolymers.

20 While free radical polymerization is useful for
polymer synthesis, several considerations limit its
suitability for use in the living animal or human body.
25 First, the initiator which generates free radicals
normally produces several small molecules with known or
unknown toxicity. For example, one of the most commonly
used photoinitiators, 2,2-dimethoxy 2-phenylacetophenone,
30 generates methyl benzoate and other small compounds
during the initiation step. The safety of these
initiator fragments must be established before there can
be widespread use of such systems for human or animal
35 use. Second, free radicals are extremely reactive
species and have life times ranging from 0.01 to 1 second
25 during a typical free radical polymerization reaction.
Third, the free radical polymerization, once initiated,
40 is often uncontrollable, frequently producing polymers
with high molecular weight and broad molecular weight
distribution. Fourth, the most common functionalities
30 used in free radical polymerization are vinylic or
acrylic, and the vinyl/acrylic polymers produced by these
45 compositions do not degrade inside the body. Fifth, free
radical polymerizable monomers often need to be inhibited
with a small amount of inhibitor to prevent the premature
50 polymerization of vinyl functionality. The most commonly
35 used inhibitors are phenols (for example, hydroquinone),

5 which are toxic and hence can be used in only limited
amounts, increasing the probability of premature
polymerization and crosslinking. Finally, free radical
10 polymerization is often exothermic, and the heat it
5 generates may cause localized burn injuries.

Electrophilic-Nucleophilic Polymerization

15 Other crosslinked polymers have been formed
using electrophilic-nucleophilic polymerization of
polymers equipped with either electrophilic or
10 nucleophilic functional groups. For example, U.S. Patent
Nos. 5,296,518 and 5,104,909 to Grasel et al. describe
20 the formation of crosslinked polymers from ethylene oxide
rich prepolymers, wherein a polyisocyanate or low
molecular weight diisocyanate is used as the
25 electrophilic polymer or crosslinker, and a
polyoxyethylene based polyol with in situ generated amine
groups is used as the nucleophilic precursor. U.S.
Patent No. 5,514,379 to Weissleder et al. describes the
30 formation of biocompatible crosslinked polymers using
20 polymeric precursors, including polyethylene glycol
derivatives, each having multiple electrophilic or
nucleophilic functional groups. U.S. Patent No.
35 5,426,148 to Tucker describes sealant compositions based
on an electrophilic-nucleophilic polymerization reaction
25 between polyether acetoacrylate and polyether amine
precursors. U.S. Patent Nos. 5,874,500 and 5,527,856 to
40 Rhee et al. also describe biocompatible crosslinked
polymers, formed from electrophilic-nucleophilic
polymerization of polymers having multiple electrophilic
30 or nucleophilic functionalities.

45 While these electrophilic-nucleophilic
polymerization methods do not suffer from the same
limitations as free radical polymerization methods,
described above, they have other limitations stemming
50 from their use of polymeric precursors. Mixing can be a
35 significant impediment to such reactions since polymeric

precursors are often of a higher viscosity and diffusion is impeded, especially with the onset of gelation. Thus, imperfections in the crosslinked structures and weaknesses may result.

In contrast, the use of at least one small molecule precursor (where small molecule refers to a molecule that is not a polymer and is typically of a molecular weight less than 2000 Daltons, or else is a polymer and is of a molecular weight of less than 1000 Daltons) allows for diffusion of the small molecule throughout the crosslinked structure, even after gelation, and thus may result in superior materials. This approach has heretofore been limited to small molecules having electrophilic end groups such as aldehyde. For example, BioGlue, marketed by Cryolife Inc., uses a glutaraldehyde-based electrophilic small molecule to react with a polymeric albumin-based nucleophilic polymer.

However, the small molecule electrophile approaches that are known suffer from several limitations. For example, glutaraldehyde is known to be a toxic compound, and in fact is used to sterilize tissues and can cause significant tissue toxicity. For isocyanate-based approaches, in order for in situ polymerization to occur without local tissue toxicity, other crosslinkers are needed. Moreover, the prior art is silent on the subject of biodegradability of these networks. This is important because in many applications it is important that the materials absorb and be cleared from the body after having served their purpose.

Visualization

As described above, advances in modern surgery provide access to the deepest internal organs with minimally invasive surgical devices. As also described above, biocompatible crosslinked polymers that can be formed in situ are useful in such surgical procedures.

5 However, most such formulations, for example, fibrin
glue, are colorless, and the amount of material used is
typically very small, leading to a film thickness of only
10 about 0.05 to 1 mm. The resulting colorless solution or
5 film is therefore difficult to visualize, especially in
the typically wet and moist surgical environment. Under
laparoscopic conditions, visibility is even more
15 difficult due to the fact that only a two-dimensional
view of the surgical field is available on the monitor
10 that is used in such procedures.

20 The use of color in biocompatible crosslinked
polymers and precursors may therefore greatly improve
their utility in a surgical environment, especially under
minimally invasive surgical procedures. Moreover, the
25 better visibility available with the use of color also
permits efficient use of materials with minimum wastage.

30 There thus exists a need for biocompatible
crosslinked polymers that can be formed without using
free radical chemistry, that can be formed from at least
20 one small molecule precursor that has minimal tissue
toxicity, that may be biodegradable, and that may be
colored.

35 Summary Of The Invention

25 It is therefore an object of the present
invention to provide biocompatible crosslinked polymers
and methods for their preparation and use, in which the
40 biocompatible crosslinked polymers are formed without
using free radical chemistry, and are formed using at
30 least one non-toxic small molecule precursor.

45 It is another object of this invention to
provide such biocompatible crosslinked polymers and
methods for their preparation and use, in which the
biocompatible crosslinked polymers are formed from
50 35 aqueous solutions, preferably under physiological
conditions.

5 It is still another object of this invention to
provide such biocompatible crosslinked polymers and
methods for their preparation and use, in which the
10 biocompatible crosslinked polymers are formed in vivo.

5 It is a still further object of this invention
to provide such biocompatible crosslinked polymers and
methods for their preparation and use, in which the
15 biocompatible crosslinked polymers are biodegradable.

Another object of this invention is to provide
10 such biocompatible crosslinked polymers and methods for
their preparation and use, in which the biocompatible
20 crosslinked polymers, their precursors, or both are
colored.

Another object of this invention is to provide
15 methods for preparing tissue conforming, biocompatible
crosslinked polymers in a desirable form, size and shape.

Another object of this invention is to provide
methods for using biocompatible crosslinked polymers to
30 form medically useful devices or implants for use as
20 surgical adhesion prevention barriers, as implantable
wound dressings, as scaffolds for cellular growth for
tissue engineering, or as surgical tissue adhesives or
35 sealants.

Another object of this invention is to provide
25 methods for using biocompatible crosslinked polymers to
form medically useful devices or implants that can
40 release bioactive compounds in a controlled manner for
local, systemic, or targeted drug delivery.

Another object of this invention is to provide
30 methods and compositions for producing composite
45 biomaterials comprising fibers or particulates made of
biodegradable biocompatible crosslinked polymers.

Brief Description Of The Drawings

50 35 FIG. 1 depicts electrophilic water soluble and
biodegradable crosslinkers or functional polymers, which

5 can be crosslinked with appropriate nucleophilic precursors.

10 FIG. 2 depicts nucleophilic water soluble and biodegradable crosslinkers or functional polymers, which
5 can be crosslinked with appropriate electrophilic precursors.

15 FIG. 3 depicts electrophilic water soluble and biodegradable crosslinkers or functional polymers, which
10 can be crosslinked with appropriate nucleophilic precursors, wherein either the biodegradable linkages or
20 the functional groups are selected so as to make the precursor water soluble.

FIG. 4 depicts nucleophilic water soluble crosslinkers or functional polymers, which can be
25 crosslinked with appropriate electrophilic precursors, and which are not biodegradable.

FIG. 5 depicts electrophilic water soluble crosslinkers or functional polymers, which can be
30 crosslinked with appropriate nucleophilic precursors, and
20 which are not biodegradable.

FIG. 6 depicts the preparation of an electrophilic water soluble crosslinker or functional
35 polymer using carbodiimide ("CDI") activation chemistry, its crosslinking reaction with a nucleophilic water
25 soluble functional polymer to form a biocompatible crosslinked polymer product, and the hydrolysis of that
40 biocompatible crosslinked polymer to yield water soluble fragments.

FIG. 7 depicts the use of sulfonyl chloride
30 activation chemistry to prepare an electrophilic functional polymer.
45

FIG. 8 depicts the preparation of an electrophilic water soluble crosslinker or functional
50 polymer using N-hydroxysuccinimide ("NHS") activation
35 chemistry, its crosslinking reaction with a nucleophilic water soluble functional polymer to form a biocompatible

crosslinked polymer product, and the hydrolysis of that biocompatible crosslinked polymer to yield water soluble fragments.

FIG. 9 depicts preferred NHS esters for use in the invention.

FIG. 10 shows the N-hydroxysulfosuccinimide ("SNHS") activation of a tetrafunctional sugar-based water soluble synthetic crosslinker and its crosslinking reaction with 4-arm amine terminated polyethylene glycol to form a biocompatible crosslinked polymer product, and the hydrolysis of that biocompatible crosslinked polymer to yield water soluble fragments.

FIG. 11 shows the variation in gelation time with the number of amino groups for the reaction of 4 arm 10 kDa succinimidyl glutarate PEG ("SG-PEG") with di-, tri- or tetra-lysine.

FIG. 12 shows the variation in gelation time with the solution age of the electrophilic functional polymer.

FIG. 13 shows the variation in gelation time with the concentration of biocompatible crosslinked polymer precursors, and with the solution age of the 4 arm 10 kDa carboxymethyl-hydroxybutyrate-N-hydroxysuccinimidyl PEG ("CM-HBA-NS") electrophilic functional polymer.

FIG. 14 shows the variation in degradation time with the concentration of biocompatible crosslinked polymer.

Detailed Description Of The Invention

The novel biocompatible crosslinked polymers of this invention are formed from the reaction of precursors having electrophilic and nucleophilic functional groups. The precursors are preferably water soluble, non-toxic and biologically acceptable.

5 Preferably, at least one of the precursors is a
small molecule, and is referred to as a "crosslinker".
10 More preferably, the crosslinker has a solubility of at
least 1 g/100 mL in an aqueous solution. Preferably, one
5 of the other precursors is a macromolecule, and is
referred to as a "functional polymer".

Functional Groups

15 Each precursor is multifunctional, meaning that
it comprises two or more electrophilic or nucleophilic
10 functional groups, such that a nucleophilic functional
group on one precursor may react with an electrophilic
20 functional group on another precursor to form a covalent
bond. At least one of the precursors comprises more than
two functional groups, so that, as a result of
15 electrophilic-nucleophilic reactions, the precursors
combine to form crosslinked polymeric products. Such
25 reactions are referred to as "crosslinking reactions".

Preferably, each precursor comprises only
30 nucleophilic or only electrophilic functional groups, so
long as both nucleophilic and electrophilic precursors
20 are used in the crosslinking reaction. Thus, for
example, if a crosslinker has nucleophilic functional
35 groups such as amines, the functional polymer may have
electrophilic functional groups such as N-
25 hydroxysuccinimides. On the other hand, if a crosslinker
has electrophilic functional groups such as
40 sulfosuccinimides, then the functional polymer may have
nucleophilic functional groups such as amines. Thus,
functional polymers such as proteins, poly(allyl amine),
30 or amine-terminated di-or multifunctional poly(ethylene
45 glycol) ("PEG") can be used.

Water Soluble Cores

The precursors preferably have biologically
inert and water soluble cores. When the core is a
50 35 polymeric region that is water soluble, preferred
polymers that may be used include: polyethers, for

5 example polyalkylene oxides such as polyethylene glycol
("PEG"), polyethylene oxide ("PEO"), polyethylene oxide-
10 co-polypropylene oxide ("PPO"), co-polyethylene oxide
block or random copolymers, and polyvinyl alcohol
5 ("PVA"); poly(vinyl pyrrolidinone) ("PVP"); poly(amino
acids); dextran and the like. The polyethers and more
particularly poly(oxyalkylenes) or poly(ethylene oxide)
15 or polyethylene oxide are especially preferred. When the
core is small molecular in nature, any of a variety of
10 hydrophilic functionalities can be used to make the
precursor water soluble. For example, functional groups
20 like hydroxyl, amine, sulfonate and carboxylate, which
are water soluble, maybe used to make the precursor water
soluble. In addition, N-hydroxysuccinimide ("NHS") ester
25 of subaric acid is insoluble in water, but by adding a
sulfonate group to the succinimide ring, the NHS ester of
subaric acid may be made water soluble, without affecting
its reactivity towards amine groups.

Biodegradable Linkages

30 20 If it is desired that the biocompatible
crosslinked polymer be biodegradable or absorbable, one
or more precursors having biodegradable linkages present
35 in between the functional groups may be used. The
biodegradable linkage optionally also may serve as the
25 water soluble core of one or more of the precursors. In
the alternative, or in addition, the functional groups of
40 the precursors may be chosen such that the product of the
reaction between them results in a biodegradable linkage.
For each approach, biodegradable linkages may be chosen
30 such that the resulting biodegradable biocompatible
45 crosslinked polymer will degrade or be absorbed in a
desired period of time. Preferably, biodegradable
linkages are selected that degrade under physiological
conditions into non-toxic products.

50 35 The biodegradable linkage may be chemically or
enzymatically hydrolyzable or absorbable. Illustrative

5 chemically hydrolyzable biodegradable linkages include
polymers, copolymers and oligomers of glycolide, dl-
10 lactide, l-lactide, caprolactone, dioxanone, and
trimethylene carbonate. Illustrative enzymatically
5 hydrolyzable biodegradable linkages include peptidic
linkages cleavable by metalloproteinases and
collagenases. Additional illustrative biodegradable
15 linkages include polymers and copolymers of poly(hydroxy
acid)s, poly(orthocarbonate)s, poly(anhydride)s,
10 poly(lactone)s, poly(aminoacid)s, poly(carbonate)s, and
poly(phosphonate)s.

20 Visualization Agents

Where convenient, the biocompatible crosslinked
polymer or precursor solutions (or both) may contain
25 visualization agents to improve their visibility during
surgical procedures. Visualization agents are especially
useful when used in MIS procedures, due among other
reasons to their improved visibility on a color monitor.

30 Visualization agents may be selected from among
20 any of the various non-toxic colored substances suitable
for use in medical implantable medical devices, such as
FD&C dyes 3 and 6, eosin, methylene blue, indocyanine
35 green, or colored dyes normally found in synthetic
surgical sutures. The preferred color is green or blue
25 because it has better visibility in presence of blood or
on a pink or white tissue background. Red is the least
40 preferred color.

The visualization agent may be present in
either a crosslinker or functional polymer solution,
30 preferably in a functional polymer solution. The
45 preferred colored substance may or may not become
incorporated into the biocompatible crosslinked polymer.
Preferably, however, the visualization agent does not
50 have a functional group capable of reacting with the
35 crosslinker or functional polymer.

5 The visualization agent may be used in small
quantities, preferably less than 1% weight/volume, more
preferably less than 0.01% weight/volume and most
10 preferably less than 0.001% weight/volume concentration.

5 Additional visualization agents may be used,
such as fluorescent (e.g., green or yellow fluorescent
under visible light) compounds (e.g., fluorescein or
15 eosin), x-ray contrast agents (e.g., iodinated compounds)
for visibility under x-ray imaging equipment, ultrasonic
20 contrast agents, or MRI contrast agents (e.g., Gadolinium
containing compounds).

20 Crosslinking Reactions

The crosslinking reactions preferably occur in
aqueous solution under physiological conditions. More
25 preferably the crosslinking reactions occur "in situ",
meaning they occur at local sites such as on organs or
tissues in a living animal or human body. More
preferably the crosslinking reactions do not release heat
30 of polymerization. Preferably the crosslinking reaction
20 leading to gelation occurs within 10 minutes, more
preferably within 2 minutes, more preferably within one
minute, and most preferably within 30 seconds.

35 Certain functional groups, such as alcohols or
carboxylic acids, do not normally react with other
25 functional groups, such as amines, under physiological
conditions (e.g., pH 7.2-11.0, 37°C). However, such
40 functional groups can be made more reactive by using an
activating group such as N-hydroxysuccinimide. Several
methods for activating such functional groups are known
30 in the art. Preferred activating groups include
45 carbonyldiimidazole, sulfonyl chloride, aryl halides,
sulfosuccinimidyl esters, N-hydroxysuccinimidyl ester,
succinimidyl ester, epoxide, aldehyde, maleimides,
imidoesters and the like. The N-hydroxysuccinimide
50 esters or N-hydroxysulfosuccinimide groups are the most
35 preferred groups for crosslinking of proteins or amine

functionalized polymers such as aminoterminated polyethylene glycol ("APEG").

FIGS. 1 to 5 illustrate various embodiments of preferred crosslinkers and functional polymers.

FIG. 1 illustrates possible configurations of degradable electrophilic crosslinkers or functional polymers. The biodegradable regions are represented by (W); the functional groups are represented by (F); and the inert water soluble cores are represented by (C). For crosslinkers, the central core is a water soluble small molecule and for functional polymers the central core is a water soluble polymer of natural or synthetic origin.

When Structure A in FIG. 1 is a functional polymer, it is a linear water soluble and biodegradable functional polymer, end-capped with two functional groups (e.g., N-hydroxysuccinimide ester or NHS, epoxide or similar reactive groups). The water soluble core may be a polyalkylene oxide, preferably polyethylene glycol block copolymer, and it is extended with at least one biodegradable linkage between it and each terminal functional group. The biodegradable linkage may be a single linkage or copolymers or homopolymers of absorbable polymers such as polyhydroxy acids or polylactones.

When Structure B in FIG. 1 is a functional polymer it is a branched or star shaped biodegradable functional polymer which has an inert polymer at the center. Its inert and water soluble core is terminated with oligomeric biodegradable extensions, which in turn are terminated with reactive functional groups.

When Structures C and D in FIG. 1 are functional polymers, they are multifunctional 4 arm biodegradable functional polymers. This polymer again has a water-soluble core at the center, which is a 4 arm, tetrafunctional polyethylene glycol (Structure C) or

5 block copolymer of PEO-PPO-PEO such as Tetronic 908
(Structure D) which is extended with by small oligomeric
10 extensions of biodegradable polymer to maintain water
solubility and terminated with reactive functional end-
5 groups such as CDI or NHS.

When Structure E in FIG. 1 is a functional
polymer, it is a multifunctional star or graft type
15 biodegradable polymer. This polymer has a water-soluble
polymer like polyethylene oxide, polyvinyl alcohol or
10 poly(vinyl pyrrolidinone) at the core which is completely
or partially extended with biodegradable polymer. The
20 biodegradable polymer is terminated with reactive end
groups.

Structures A-E in FIG. 1 need not have
25 polymeric cores and may be small molecule crosslinkers.
In that case, the core may comprise a small molecule like
ethoxylated glycerol, inositol, trimethylolpropane etc.
to form the resultant crosslinker. In addition,
30 Structures A-E in FIG. 1 need not have polymeric
20 biodegradable extensions, and the biodegradable
extensions may consist of small molecules like succinate
or glutarate or combinations of 2 or more esters, such as
35 glycolate/2-hydroxybutyrate or glycolate/4-
hydroxyproline, etc. A dimer or trimer of 4-
25 hydroxyproline may be used not only to add degradability,
but also to add nucleophilic reactive sites via the
40 pendant primary amines which are part of the
hydroxyproline moiety.

Other variations of the core, the biodegradable
30 linkage, and the terminal electrophilic group in
45 Structures A-E in FIG. 1 may be constructed, so long as
the resulting functional polymer has the properties of
low tissue toxicity, water solubility, and reactivity
with nucleophilic functional groups.

50 35 FIG. 2 illustrates various embodiments of
nucleophilic biodegradable water-soluble crosslinkers and

functional polymers suitable for use with electrophilic functional polymers and crosslinkers described herein.

The biodegradable regions are represented by (~~~~~); the functional groups are represented by (~~~~~); and the inert water soluble cores are represented by (—). For crosslinkers, the central core is a water soluble small molecule and for functional polymers the central core is a water soluble polymer of natural or synthetic origin.

When Structure F in FIG. 2 is a functional polymer, it is a linear water-soluble biodegradable polymer terminated with reactive functional groups like primary amine. The linear water-soluble core is a polyalkylene oxide, preferably polyethylene glycol block copolymer, which is extended with the biodegradable region which is a copolymer or homopolymer of polyhydroxy acids or polylactones. This biodegradable polymer is terminated with primary amines.

When Structure G in FIG. 2 is a functional polymer, it is a branched or star shaped biodegradable polymer which has an inert polymer at the center. The inert polymer is extended with single or oligomeric biodegradable extensions which are terminated with reactive functional groups.

When Structures H and I in FIG. 2 are functional polymers, they are multifunctional 4 arm biodegradable polymers. These polymers again have water-soluble cores at their center which are either a 4 arm, tetrafunctional polyethylene glycol (Structure H) or a block copolymer of PEO-PPO-PEO such as Tetronic 908 (Structure I), extended with small oligomeric extensions of biodegradable polymers to maintain water solubility, and terminated with functional groups such as amines and thiols.

When Structure J in FIG. 2 is a functional polymer, it is a multifunctional star or graft type

5 biodegradable polymer. This polymer has a water-soluble
polymer like polyethylene oxide, polyvinyl alcohol or
10 poly(vinyl pyrrolidinone) at the core which is completely
or partially extended with biodegradable polymer. The
5 biodegradable polymer is terminated with reactive end
groups.

15 Structures F-J in FIG. 2 need not have
polymeric cores and may be small molecule crosslinkers.
In that case, the core may comprise a small molecule like
10 ethoxylated glycerol, inositol, trimethylolpropane etc.
to form the resultant crosslinker.

20 Other variations of the core, the biodegradable
linkage, and the terminal nucleophilic group in
Structures F-J in FIG. 2 may be constructed, so long as
25 the resulting functional polymer has the properties of
low tissue toxicity, water solubility, and reactivity
with electrophilic functional groups.

30 FIG. 3 illustrates configurations of water
soluble electrophilic crosslinkers or functional polymers
20 where the core is biodegradable. The biodegradable
regions are represented by (www) and the functional
groups are represented by (▶). The biodegradable core
35 is terminated with a reactive functional group that is
also water solubilizing, such a N-hydroxysulfosuccinimide
25 ester ("SNHS") or N-hydroxyethoxylated succinimide ester
("ENHS").

40 Structure K in FIG. 3 depicts a difunctional
biodegradable polymer or oligomer terminated with SNHS or
ENHS. The oligomers and polymers may be made of a
45 30 poly(hydroxy acid) such as poly(lactic acid), which is
insoluble in water. However, the terminal carboxylic
acid group of these oligomers or polymers can be
activated with N-hydroxysulfosuccinimide ester ("SNHS")
50 or N-hydroxyethoxylated succinimide ester ("ENHS")
35 groups. An ionic group, like a metal salt (preferably

5 sodium salt) of sulfonic acid, or a nonionic group, like
a polyethylene oxide on the succinimide ring, provides
10 water solubility while the NHS ester provides chemical
reactivity towards amines. The sulfonate groups (sodium
5 salts) or ethoxylated groups on the succinimide ring
solubilize the oligomer or polymer without appreciably
inhibiting reactivity towards amine groups.

15 Structures L-O in FIG. 3 represent multi-
branched or graft type structures with terminal SNHS or
10 ENHS group. The cores may comprise various non-toxic
polyhydroxy compounds like sugars (xylitol, erythritol),
20 glycerol, trimethylolpropane, which have been reacted
with anhydrides such as succinic or glutaric anhydrides.
The resultant acid groups were then activated with SNHS
15 or ENHS groups to form water-soluble crosslinkers or
functional polymers.

FIG. 4 illustrates various nucleophilic
functional polymers or crosslinkers that are not
30 biodegradable. The nucleophilic functional groups are
represented by (|||||) and the inert water soluble cores
20 are represented by (—). For crosslinkers, the central
core is a water soluble small molecule and for functional
35 polymers the central core is a water soluble polymer of
natural or synthetic origin.

25 When Structure P in FIG. 4 is a functional
polymer it may be a water-soluble linear polymer such as
40 polyethylene glycol terminated with reactive end group
such as primary amines and thiols. Such polymers are
commercially available from Sigma (Milwaukee, WI) and
45 Shearwater Polymers (Huntsville, AL). Some other
preferred difunctional polymers are PPO-PEO-PPO block
copolymers such as Pluronic F68 terminated with amine
groups. Pluronic or Tetronic polymers are normally
50 available with terminal hydroxyl groups. The hydroxyl
35 groups are converted into amine groups by methods known

in the art.

When Structures Q-T in FIG. 4 are functional polymers they may be multifunctional graft or branch type water-soluble copolymers with terminal amine groups.

Structures P-T in FIG. 4 need not have polymeric cores and may be small molecule crosslinkers. In that case, the core may comprise a small molecule like ethoxylated glycerol, inositol, trimethylolpropane, dilysine etc. to form the resultant crosslinker.

Other variations of the core and the terminal nucleophilic group in Structure P-T in FIG. 4 may be employed, so long as the properties of low tissue toxicity, water solubility, and reactivity with electrophilic functional groups are maintained.

FIG. 5 illustrates various electrophilic functional polymers or crosslinkers that are not biodegradable. The electrophilic functional groups are represented by (▶) and the inert water soluble cores are represented by (—). For crosslinkers, the central core is a water soluble small molecule and for functional polymers the central core is a water soluble polymer of natural or synthetic origin.

When Structure U is a functional polymer, it may be a water-soluble polymer such as polyethylene glycol terminated reactive end group such as NHS or epoxide. Such polymers are commercially available from Sigma and Shearwater polymers. Some other preferred polymers are PPO-PEO-PPO block copolymers such as Pluronic F68 terminated with NHS or SNHS group. Pluronic or Tetronic polymers are normally available with terminal hydroxyl groups. The hydroxyl groups are converted into acid group by reacting with succinic anhydride. The terminated acid groups are reacted with N-hydroxysuccinimide in presence of DCC to generate NHS activated Pluronic polymer.

When Structures V-Y are functional polymers they may be multifunctional graft or branch type PEO or PEO block copolymers (Tetronics) activated with terminal reactive groups such as NHS.

Structures U-Y in FIG. 5 need not have polymeric cores and may be small molecule crosslinkers. In that case, the core may comprise a small molecule like ethoxylated glycerol, inositol, trimethylolpropane, dilysine etc. to form the resultant crosslinker.

Other variations of the core and the terminal nucleophilic group in Structures U-Y in FIG. 5 may be employed, so long as the properties of low tissue toxicity, water solubility, and reactivity with electrophilic functional groups are maintained.

Preparation of Structures A-Y in FIGS. 1-5
The polymeric crosslinkers and functional polymers illustrated as Structures A-Y in FIGS. 1 to 5 may be prepared using variety of synthetic methods. Their preferred compositions are described in Table 1.

Table 1.
Preferred Crosslinkers and Functional Polymers

Structure	Brief Description	Typical Example
A	Water soluble, linear difunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences which are cleavable by enzymes and terminated with protein reactive functional groups.	Polyethylene glycol or ethoxylated propylene glycol chain extended with oligolactate and terminated with N-hydroxysuccinimide esters.

Structure	Brief Description	Typical Example
B	Water soluble, trifunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with protein reactive functional groups	Ethoxylated glycerol chain extended with oligolactate and terminated with N-hydroxysuccinimide esters
C	Water soluble, tetrafunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with protein reactive functional groups	4 arm polyethylene glycol, erythritol or pentaerythritol chain extended with oligolactate and terminated with N-hydroxysuccinimide esters
D	Water soluble, tetrafunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with protein reactive functional groups	Ethoxylated ethylene diamine or polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer like Tetronic 908 chain extended with oligotrimethylene carbonate and terminated with N-hydroxysuccinimide ester
E	Water soluble, branched crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with protein reactive functional groups	Low molecular weight polyvinyl alcohol with 1% to 20% hydroxyl groups extended with oligolactate and terminated with N-hydroxysuccinimide ester

Structure	Brief Description	Typical Example
F	Water soluble, linear difunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	Polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer surfactant like Pluronic F68 chain extended with oligolactate and terminated with amino acids such as lysine or peptide sequences that may contain two amine groups
G	Water soluble, trifunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	Ethoxylated glycerol chain extended with oligolactate and terminated with amino acid such as lysine
H	Water soluble, tetrafunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	4 arm polyethylene glycol or tetra erythritol chain extended with oligolactate and terminated with amino acid such as lysine
I	Water soluble, tetrafunctional crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	Ethoxylated ethylene diamine or polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer like Tetronic 908 chain extended with oligotrimethylene carbonate and terminated with amino acid such as lysine

Structure	Brief Description	Typical Example
J	Water soluble, multifunctional or graft type crosslinker or functional polymer with water soluble core, extended with biodegradable regions such as oligomers of hydroxyacids or peptide sequences and terminated with amines, carboxylic acid or thiols	Low molecular weight polyvinyl alcohol with 1-20% hydroxyl groups extended with oligolactate and terminated with aminoacid such as lysine
K	Water soluble, linear difunctional crosslinker or functional polymer such as oligomers of hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Difunctional oligolactic acid with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimide ester or ethoxylated n-hydroxysuccinimide ester.
L	Water soluble branched trifunctional crosslinker or functional polymer such as oligomers of hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Trifunctional oligocaprolactone with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimide ester or ethoxylated n-hydroxysuccinimide ester.
M	Water soluble, branched tetrafunctional crosslinker or functional polymer such as oligomers of hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Tetrafunctional oligocaprolactone with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimide ester or ethoxylated n-hydroxysuccinimide ester.

Structure	Brief Description	Typical Example
N	Water soluble, branched tetrafunctional crosslinker or functional polymer such as oligomers of hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Tetrafunctional oligocaprolactone with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimide ester or ethoxylated n-hydroxysuccinimide ester.
O	Water soluble, branched multifunctional crosslinker or functional polymer such as oligomers of hydroxyacids or peptide sequences which are terminated with protein reactive functional groups	Multifunctional oligolactic acid with terminal carboxyl groups which are activated with n-hydroxysulfosuccinimide ester or ethoxylated n-hydroxysuccinimide ester.
P	Water soluble, linear difunctional crosslinker or functional polymer terminated with amines, carboxylic acid or thiols functional groups	Polyethylene glycol with terminal amines groups
Q	Water soluble, branched trifunctional crosslinker or functional polymer terminated with amines, carboxylic acid or thiols as functional group	Ethoxylated glycerol with terminal amines groups
R	Water soluble, branched tetrafunctional crosslinker or functional polymer terminated with amines, carboxylic acid or thiols functional groups	4 arm polyethylene glycol modified to produce terminal amine groups

Structure	Brief Description	Typical Example
S	Water soluble, branched tetrafunctional crosslinker or functional polymer terminated with amines, carboxylic acid or thiols functional groups	Ethoxylated ethylene diamine or polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer like Tetronic 908 modified to generate terminal amine groups
T	Water soluble, branched or graft crosslinker or functional polymer with terminal amines, carboxylic acid or thiols functional groups	Polylysine, albumin, polyallyl amine
U	Water soluble, linear difunctional crosslinker or functional polymer terminated with protein reactive functional groups	Polyethylene glycol with n-hydroxysuccinimide as end groups
V	Water soluble branched trifunctional crosslinker or functional polymer terminated with protein reactive functional groups	Ethoxylated glycerol terminated with n-hydroxysuccinimide
W	Water soluble branched tetrafunctional crosslinker or functional polymer terminated with protein reactive functional groups	4 arm polyethylene glycol terminated with n-hydroxysuccinimide esters
X	Water soluble branched tetrafunctional crosslinker or functional polymer terminated with protein reactive functional groups	Ethoxylated ethylene diamine or polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer like Tetronic 908 with n-hydroxysuccinimide ester as end group

Structure	Brief Description	Typical Example
Y	Water soluble, branched or graft polymer crosslinker or functional polymer with protein reactive functional groups	Poly(vinyl pyrrolidinone)-co-poly(n-hydroxysuccinimide acrylate) copolymer (9:1), molecular weight < 40000 Da

First, the biodegradable links of Structures A-J in FIGS. 1 and 2 may be composed of specific di or multifunctional synthetic amino acid sequences which are recognized and cleaved by enzymes such as collagenase, and may be synthesized using methods known to those skilled in the peptide synthesis art. For example, Structures A-E in FIG. 1 may be obtained by first using carboxyl, amine or hydroxy terminated polyethylene glycol as a starting material for building a suitable peptide sequence. The terminal end of the peptide sequence is converted into a carboxylic acid by reacting succinic anhydride with an appropriate amino acid. The acid group generated is converted to an NHS ester by reaction with N-hydroxysuccinimide.

The functional polymers described in FIG. 2 may be prepared using a variety of synthetic methods. In a preferred embodiment, the polymer shown as Structure F may be obtained by ring opening polymerization of cyclic lactones or carbonates initiated by a dihydroxy compound such as Pluronic F 68 in the presence of a suitable catalyst such as stannous 2-ethylhexanoate. The molar equivalent ratio of caprolactone to Pluronic is kept below 10 to obtain a low molecular weight chain extension product so as to maintain water solubility. The terminal hydroxyl groups of the resultant copolymer are converted into amine or thiol by methods known in the art.

In a preferred method, the hydroxyl groups of a Pluronic-caprolactone copolymer are activated using tresyl chloride. The activated groups are then reacted with lysine to produce lysine terminated Pluronic-

5 caprolactone copolymer. Alternatively, an amine-blocked
lysine derivative is reacted with the hydroxyl groups of
10 a Pluronic-caprolactone copolymer and then the amine
groups are regenerated using a suitable deblocking
5 reaction.

Structures G, H, I and J in FIG. 2 may
represent multifunctional branched or graft type
15 copolymers having water-soluble core extended with
oligohydroxy acid polymer and terminated with amine or
10 thiol groups.

For example, in a preferred embodiment, the
functional polymer illustrated as Structure G in FIG. 2
is obtained by ring opening polymerization of cyclic
lactones or carbonates initiated by a tetrahydroxy
25 compound such as 4 arm, tetrahydroxy polyethylene glycol
(molecular weight 10,000 Da), in the presence of a
suitable catalyst such as stannous octoate. The molar
equivalent ratio of cyclic lactone or carbonate to PEG is
30 kept below 10 to obtain a low molecular weight extension,
and to maintain water solubility (polymers of cyclic
lactones generally are not as water soluble as PEG).
Alternatively, hydroxyacid as a biodegradable link may be
35 attached to the PEG chain using blocking/deblocking
chemistry known in the peptide synthesis art. The
terminal hydroxy groups of the resultant copolymer are
25 activated using a variety of reactive groups known in the
art. The CDI activation chemistry and sulfonyl chloride
activation chemistry is shown in FIGS. 6 and 7,
40 respectively.

30 The most preferred reactive groups are
N-hydroxysuccinimide esters, synthesized by any of
several methods. In a preferred method, hydroxyl groups
are converted to carboxylic groups by reacting them with
45 anhydrides such as succinic anhydride in the presence of
tertiary amines such as pyridine or triethylamine or
50 dimethylaminopyridine ("DMAP"). Other anhydrides such as

5 glutaric anhydride, phthalic anhydride, maleic anhydride
and the like may also be used. The resultant terminal
10 carboxyl groups are reacted with N-hydroxysuccinimide in
the presence of dicyclohexylcarbodiimide ("DCC") to
5 produce N-hydroxysuccinimide ester (referred as NHS
activation). The NHS activation and crosslinking
reaction scheme is shown in FIG. 8. The most preferred
15 N-hydroxysuccinimide esters are shown in FIG. 9.

In a preferred embodiment, the polymer shown as
10 structure H is obtained by ring opening polymerization of
glycolide or trimethylene carbonate initiated by a
20 tetrahydroxy compound such as tetrafunctional
polyethylene glycol (molecular weight 2000 Da) in the
presence of a catalyst such as stannous 2-ethylhexoate.
25 The molar equivalent ratio of glycolide to PEG is kept
from 2 to 10 to obtain a low molecular weight extension.
The terminal hydroxy groups of the resultant copolymer
are converted into amine groups by reaction with lysine
30 as mentioned previously. Similar embodiments can be
20 obtained using analogous chain extension synthetic
strategies to obtain structures F, G, I and J by starting
with the appropriate corresponding polyol.

35 Structures K, L, M, N, and O in FIG. 3 are made
using a variety of synthetic methods. In a preferred
25 embodiment, the polymer shown as Structure L in FIG. 3 is
obtained by ring opening polymerization of cyclic
40 lactones by a trihydroxy compound such as glycerol in the
presence of a catalyst such as stannous 2-ethylhexanoate.
The molar equivalent ratio of cyclic lactone to glycerol
30 is kept below 2, so that only low molecular weight
45 oligomers are obtained. The low molecular weight
oligomer ester is insoluble in water. The terminal
hydroxy groups of the resultant copolymer are activated
50 using N-hydroxysulfosuccinimide groups. This is achieved
35 by converting hydroxy groups to carboxylic groups by
reacting with anhydrides such as succinic anhydride in

5 presence of tertiary amines. The resultant terminal
carboxyl groups are reacted with N-
10 hydroxysulfosuccinimide or N-hydroxyethoxylated
succinimide in the presence of dicyclohexylcarbodiimide
5 ("DCC") to produce a sulfonated or ethoxylated NHS ester.
The sulfonate or PEO chain on the succinimide ring gives
water solubility to the oligoester.

15 The foregoing method generally is applied to
solubilize only low molecular weight multi-branched
10 oligoesters, with molecular weights below 1000. In
another variation of this method, various non-toxic
20 polyhydroxy compounds, preferably sugars, such as
erythritol, xylitol are reacted with succinic anhydride
in the presence of a tertiary amine. The terminal
15 carboxyl group of succinated erythritol is esterified
with N-hydroxysulfosuccinimide (FIG. 9). Similar
25 embodiments may be obtained using analogous synthetic
strategies to obtain structures K, and M-O by starting
with the appropriate starting materials.

30 20 Structures P-R may be synthesized by reacting
the appropriate starting material, such as a linear (P)
or 2- or 3-arm branched PEG (Q, R) with hydroxy end
35 groups, with lysine as mentioned previously, such that
the arms of the PEG oligomers are capped with amine end
25 groups. Structure S may be synthesized, using a
multistep reaction, from PEG, glycerol and a
40 diisocyanate. In the first step a PEG diol is reacted
with excess diisocyanate, such as 4,4'diphenyl methane
diisocyanate ("MDI"), methylene-bis(4-
30 cyclohexylisocyanate) ("HMDI") or
45 hexamethylenediisocyanate ("HDI"). After purification
the resultant PEG diisocyanate is added dropwise to
excess glycerol or trimethylol propane or other triol and
reacted to completion. The purified product, now having
50 35 diol end groups, is again reacted with excess
diisocyanate and purified, yielding a PEG-tetra-

5 isocyanate. This tetrafunctional PEG subsequently may be
reacted with excess PEG diols, yielding a 4 arm PEG
10 synthesized from a PEG diol oligomer. In the final step
lysine end groups are incorporated, as discussed
5 previously.

Structure T may be synthesized as follows.

15 First synthesize a random copolymer of PEG-monoacrylate
and some other acrylate or combination of acrylates, such
that the final polyacrylate is water soluble. Other
10 acrylates include, but are not limited to, 2-
hydroxyethylacrylate, acrylic acid, and acrylamide.
20 Conditions may be varied to control the molecular weight
as desired. In the final step, the acrylate is reacted
with lysine as discussed previously, using an appropriate
15 quantity to achieve the desired degree of amination.

25 One method of synthesizing Structures U-Y is to
use dicyclohexylcarbodiimide coupling to a carboxylate
end group. For Structures U-W, one can react the
30 appropriate PEG-diol, -triol or -tetra-hydroxy starting
20 material with excess succinic anhydride or glutaric
anhydride such that all end groups are effectively
carboxylated. Structures X and Y may be made in a manner
35 similar to that used for Structures S and T, except that
in the last step, instead of end capping with lysine, end
25 capping with succinic anhydride or glutaric anhydride is
performed.

40 Preparation of Biocompatible Polymers

Several biocompatible crosslinked polymers may
be produced using the crosslinkers and functional
30 polymers described in FIGS. 1 to 5. Preferred
45 combinations of such polymers suitable for producing such
biocompatible crosslinked polymers are described in Table
1 and Table 2. In Table 2, the crosslinker functional
groups are N-hydroxy succinimide esters and the
50 functional polymer functional groups are primary amines.
35

Table 2.
Biocompatible Polymers Synthesized from
Crosslinkers and Functional Polymers Of Table 1

Crosslinker Structure	Functional Polymer Structure	Concentration	Medium
B or C	H and R	Molar Equivalent; > 20% W/V	Borate or triethanol amine buffer, pH 7-9
A, B or C	H, P, Q, R and S	Molar Equivalent; > 20% W/V	Borate or triethanol amine buffer, pH 7-9
Y	T, H, P and Q	Molar Equivalent; > 10 % W/V	Borate or triethanol amine buffer, pH 7-9
W, V	H and J	Molar Equivalent; > 10 % W/V	Bicarbonate buffer, pH 9
X	I, J and H	Molar Equivalent; > 20% W/V	Borate or triethanol amine buffer, pH 7-9

The reaction conditions for crosslinking will depend on the nature of the functional groups. Preferred reactions are conducted in buffered aqueous solutions at pH 5 to 12. The preferred buffers are sodium borate buffer (pH 10) and triethanol amine buffer (pH 7). In some embodiments, organic solvents such as ethanol or isopropanol may be added to improve the reaction speed or to adjust the viscosity of a given formulation.

The synthetic crosslinked gels described above degrade due to hydrolysis of the biodegradable region. The degradation of gels containing synthetic peptide sequences will depend on the specific enzyme and its concentration. In some cases, a specific enzyme may be added during the crosslinking reaction to accelerate the degradation process.

When the crosslinker and functional polymers are synthetic (for example, when they are based on

5 polyalkylene oxide), then it is desirable and in some cases essential to use molar equivalent quantities of the reactants. In some cases, molar excess crosslinker may
10 be added to compensate for side reactions such as
5 reactions due to hydrolysis of the functional group.

When choosing the crosslinker and crosslinkable polymer, at least one of polymers must have more than 2
15 functional groups per molecule and at least one degradable region, if it is desired that the resultant
10 biocompatible crosslinked polymer be biodegradable. For example, the difunctional crosslinker shown as Structure A in FIG. 1 cannot form a crosslinked network with the difunctional polymers shown as Structure F in FIG. 2 or Structure P in Fig. 4. Generally, it is preferred that
20 each biocompatible crosslinked polymer precursor have
25 more than 2 and more preferably 4 functional groups.

Preferred electrophilic groups are NHS, SNHS and ENHS (FIG. 9). Preferred nucleophilic groups are primary amines. The advantage of the NHS-amine reaction
30 is that the reaction kinetics lead to quick gelation usually within 10 minutes, more usually within 1 minute and most usually within 10 seconds. This fast gelation
35 is preferred for in situ reactions on live tissue.

The NHS-amine crosslinking reaction leads to
25 formation of N-hydroxysuccinimide as a side product. The sulfonated or ethoxylated forms of N-hydroxysuccinimide
40 are preferred due to their increased solubility in water and hence their rapid clearance from the body. The sulfonic acid salt on the succinimide ring does not alter
30 the reactivity of NHS group with the primary amines.

45 The NHS-amine crosslinking reaction may be carried out in aqueous solutions and in the presence of buffers. The preferred buffers are phosphate buffer (pH 5.0-7.5), triethanolamine buffer (pH 7.5-9.0) and
50 borate buffer (pH 9.0-12) and sodium bicarbonate buffer (pH 9.0-10.0).
55

5 Aqueous solutions of NHS based crosslinkers and
functional polymers preferably are made just before the
crosslinking reaction due to reaction of NHS groups with
10 water. Longer "pot life" may be obtained by keeping
5 these solutions at lower pH (pH 4-5).

The crosslinking density of the resultant
biocompatible crosslinked polymer is controlled by the
15 overall molecular weight of the crosslinker and
functional polymer and the number of functional groups
20 available per molecule. A lower molecular weight between
crosslinks such as 600 Da will give much higher
crosslinking density as compared to a higher molecular
weight such as 10,000 Da. Higher molecular weight
functional polymers are preferred, preferably more than
25 3000 Da, so as to obtain elastic gels.

The crosslinking density also may be controlled
by the overall percent solids of the crosslinker and
functional polymer solutions. Increasing the percent
30 solids increases the probability that an electrophilic
group will combine with a nucleophilic group prior to
inactivation by hydrolysis. Yet another method to
control crosslink density is by adjusting the
stoichiometry of nucleophilic groups to electrophilic
35 groups. A one to one ratio leads to the highest
25 crosslink density.

Preparation of Biodegradable Polymers

40 The biodegradable crosslinkers described in
FIGS. 1 and 3 may be reacted with proteins, such as
albumin, other serum proteins, or serum concentrates to
30 generate crosslinked polymeric networks. Briefly,
45 aqueous solutions of the crosslinkers described in FIG. 1
and FIG. 3 (at a concentration of 50 to 300 mg/ml) are
mixed with concentrated solutions of albumin (600 mg/ml)
to produce a crosslinked hydrogel. This reaction can be
50 accelerated if a buffering agent, e.g., borate buffer or
35 triethanol amine, is added during the crosslinking step.

5 The resultant crosslinked hydrogel is a
semisynthetic hydrogel whose degradation depends on the
degradable segment in the crosslinker as well as
10 degradation of albumin by enzymes. In the absence of any
5 degradable enzymes, the crosslinked polymer will degrade
solely by the hydrolysis of the biodegradable segment.
If polyglycolate is used as the biodegradable segment,
15 the crosslinked polymer will degrade in 1-30 days
depending on the crosslinking density of the network.
10 Similarly, a polycaprolactone based crosslinked network
will degrade in 1-8 months. The degradation time
20 generally varies according to the type of degradable
segment used, in the following order: polyglycolate <
polylactate < polytrimethylene carbonate <
15 polycaprolactone. Thus, it is possible to construct a
25 hydrogel with a desired degradation profile, from a few
days to months, using a proper degradable segment.

 The hydrophobicity generated by biodegradable
30 blocks such as oligohydroxy acid blocks or the
20 hydrophobicity of PPO blocks in Pluronic or Tetronic
polymers are helpful in dissolving small organic drug
molecules. Other properties which will be affected by
35 incorporation of biodegradable or hydrophobic blocks are:
water absorption, mechanical properties and
25 thermosensitivity.

Methods of Using Biocompatible Polymers

40 The biocompatible crosslinked polymers and
their precursors described above may be used in a variety
of applications, such as components of tissue adhesives,
30 tissue sealants, drug delivery vehicles, wound covering
45 agents, barriers in preventing postoperative adhesions,
and others. These and other suitable applications are
reviewed in Schlag and Redl, "Fibrin Sealant" in
Operative Surgery, volumes 1-7 (1986), which is
50 incorporated herein by reference.

In Situ Formation

In many applications, the biocompatible crosslinked polymers of this invention typically will be formed "in situ" at a surgical site in the body. The various methodologies and devices for performing "in situ" gelation, developed for other adhesive or sealant systems such as fibrin glue or sealant applications, may be used with the biocompatible crosslinked polymers of this invention. Thus, in one embodiment, an aqueous solution of a freshly prepared crosslinker (e.g., SNHS-terminated oligolactide synthesized from a glycerol core in phosphate buffered saline ("PBS") at pH 5 to 7.2) and a functional polymer (e.g., albumin or amine terminated tetrafunctional polyethylene glycol at pH 10 in sodium borate) are applied and mixed on the tissue using a double barrel syringe (one syringe for each solution). The two solutions may be applied simultaneously or sequentially. In some embodiments, it is preferred to apply the precursor solutions sequentially so as to "prime" the tissue, resulting in improved adherence of the biocompatible crosslinked polymer to the tissue. Where the tissue is primed, the crosslinker precursor is preferably applied to the tissue first, followed by the functional polymer solution.

One may use specialized devices to apply the precursor solutions, such as those described in U.S. Patent Nos. 4,874,368; 4,631,055; 4,735,616; 4,359,049; 4,978,336; 5,116,315; 4,902,281; 4,932,942; Published Patent Cooperation Treaty Patent Application No. WO 91/09641; and R.A. Tange, "Fibrin Sealant" in Operative Medicine: Otolaryngology, volume 1 (1986), the disclosures of which are herein incorporated by reference.

Drug Delivery

The subject crosslinkers, functional polymer and their reaction products, the crosslinked materials

5 advantageously may be used for localized drug therapy.
Biologically active agents or drug compounds that may be
10 added and delivered from the crosslinked polymer or gel
include: proteins, glycosaminoglycans, carbohydrates,
5 nucleic acid, inorganic and organic biologically active
compounds where specific biologically active agents
include but are not limited to: enzymes, antibiotics,
15 antineoplastic agents, local anesthetics, hormones,
angiogenic agents, anti-angiogenic agents, growth
10 factors, antibodies, neurotransmitters, psychoactive
drugs, anticancer drugs, chemotherapeutic drugs, drugs
20 affecting reproductive organs, genes, and
oligonucleotides.

To prepare such crosslinked composition, the
15 bioactive compounds described above are mixed with the
crosslinkable polymer prior to making the aqueous
solution or during the aseptic manufacturing of the
functional polymer. This mixture then is mixed with the
30 crosslinker to produce a crosslinked material in which
20 the biologically active substance is entrapped.
Functional polymers made from inert polymers like
Pluronic, Tetronics or Tween™ surfactants are preferred
35 in releasing small molecule hydrophobic drugs.

In a preferred embodiment, the active agent or
25 agents are present in a separate phase when crosslinker
and crosslinkable polymers are reacted to produce a
40 crosslinked polymer network or gel. This phase
separation prevents participation of bioactive substance
in the chemical crosslinking reaction such as reaction
30 between NHS ester and amine group. The separate phase
45 also helps to modulate the release kinetics of active
agent from the crosslinked material or gel, where
'separate phase' could be oil (oil-in water emulsion),
biodegradable vehicle; and the like. Biodegradable
50 vehicles in which the active agent may be present
include: encapsulation vehicles, such as microparticles,

5 microspheres, microbeads, micropellets, and the like,
where the active agent is encapsulated in a bioerodable
or biodegradable polymers such as polymers and copolymers
10 of: poly(anhydride), poly(hydroxy acid)s, poly(lactone)s,
5 poly(trimethylene carbonate), poly(glycolic acid),
poly(lactic acid), poly(glycolic acid)-co-poly(glycolic
acid), poly(orthocarbonate), poly(caprolactone),
15 crosslinked biodegradable hydrogel networks like fibrin
glue or fibrin sealant, caging and entrapping molecules,
10 like cyclodextrin, molecular sieves and the like.
Microspheres made from polymers and copolymers of
20 poly(lactone)s and poly(hydroxy acid) are particularly
preferred as biodegradable encapsulation vehicles.

In using crosslinked materials which are
25 described herein as drug delivery vehicles, the active
agent or encapsulated active agent may be present in
solution or suspended form in crosslinker component or
functional polymer solution component. The nucleophilic
30 component, whether it be in the crosslinker or the
20 functional polymer is the preferred vehicle due to
absence of reactive groups. The functional polymer along
with bioactive agent, with or without encapsulating
35 vehicle, is administered to the host along with
equivalent amount of crosslinker and aqueous buffers.
25 The chemical reaction between crosslinker and the
functional polymer solution readily takes place to form a
40 crosslinked gel and acts as a depot for release of the
active agent to the host. Such methods of drug delivery
find use in both systemic and local administration of an
30 active agent.

45 In using the crosslinked composition for drug
delivery as mentioned above, the amount of crosslinkable
polymer, crosslinker and the dosage agent introduced in
the host will necessarily depend upon the particular drug
50 35 and the condition to be treated. Administration may be
by any convenient means such as syringe, canula, trocar,

5 catheter and the like.

Controlled rates of drug delivery also may be
obtained with the system of the present invention by
10 degradable, covalent attachment of the bioactive
5 molecules to the crosslinked hydrogel network. The
nature of the covalent attachment can be controlled to
enable control of the release rate from hours to weeks or
15 longer. By using a composite made from linkages with a
range of hydrolysis times, a controlled release profile
10 may be extended for longer durations.

20 Composite Biomaterials

The biocompatible crosslinked polymers of this
invention optionally may be reinforced with flexible or
rigid fibers, fiber mesh, fiber cloth and the like. The
15 insertion of fibers improves mechanical properties like
flexibility, strength, and tear resistance. In
implantable medical applications, biodegradable fibers,
cloth, or sheets made from oxidized cellulose or
30 poly(hydroxy acid)s polymers like polylactic acid or
20 polyglycolic acid, are preferred. Such reinforced
structures may be produced using any convenient protocol
known in the art.

35 In a preferred method, aqueous solutions of
functional polymers and crosslinkers are mixed in
25 appropriate buffers and proportions are added to a fiber
cloth or net such as Interceed (Ethicon Inc., New
40 Brunswick, NJ). The liquid mixture flows into the
interstices of the cloth and becomes crosslinked to
produce a composite hydrogel. Care is taken to ensure
30 that the fibers or fiber mesh are buried completely
45 inside the crosslinked hydrogel material. The composite
structure can be washed to remove side products such as
N-hydroxysuccinimide. The fibers used are preferably
50 hydrophilic in nature to ensure complete wetting of the
35 fibers by the aqueous gelling composition.

EXAMPLES

The following non-limiting examples are intended to illustrate the synthesis of new biocompatible crosslinked polymers and their precursors, and their use in making several medical products. Those skilled in the art will appreciate that modifications can be made to these examples, drawings, illustrations and claims that are intended to fall within the scope the present invention.

Materials and Equipment

Polyethylene glycol was purchased from various sources such as Shearwater Polymers, Union Carbide, Fluka and Polysciences. Multifunctional hydroxyl and amine terminated polyethylene glycol were purchased from Shearwater Polymers, Dow Chemicals and Texaco. Pluronic® and Tetronic® series polyols were purchased from BASF Corporation. DL-lactide, glycolide, caprolactone and trimethylene carbonate was obtained from commercial sources like Purac, DuPont, Polysciences, Aldrich, Fluka, Medisorb, Wako and Boehringer Ingelheim. N-hydroxysulfosuccinimide was purchased from Pierce. All other reagents, solvents were of reagent grade and were purchased from commercial sources such as Polysciences, Fluka, Aldrich and Sigma. Most of the reagents and solvents were purified and dried using standard laboratory procedures such as described in D.D. Perrin et al., Purification of Laboratory Chemicals (Pergamon Press 1980).

General Analysis

The polymers synthesized according to these examples were chemically analyzed using structure-determining methods such as nuclear (proton and carbon-13) magnetic resonance spectroscopy, infrared spectroscopy. Molecular weights were determined using high pressure liquid chromatography and gel permeation chromatography. Thermal characterization of the

5 polymers, including melting point and glass transition
temperatures, were performed using differential scanning
10 calorimetric analysis. Aqueous solution properties such
as micelle and gel formation was determined using
5 fluorescence spectroscopy, UV-visible spectroscopy and
laser light scattering instruments.

15 In vitro degradation of the polymers was
followed gravimetrically at 37 °C, in an aqueous buffered
medium such as phosphate buffered saline (at pH 7.2). In
10 vivo biocompatibility and degradation life times was
assessed by injecting or forming a gelling formulation
20 directly into the peritoneal cavity of a rat or rabbit
and observing its degradation over a period of 2 days to
12 months.

25 Alternatively, the degradation was also
assessed by prefabricating a sterile implant, made by a
process like solution casting, then surgically implanting
the implant within an animal body. The degradation of
30 the implant over time was monitored gravimetrically or by
20 chemical analysis. The biocompatibility of the implant
was assessed by standard histological techniques.

35 **Example 1.** Synthesis of a water-soluble difunctional,
biodegradable functional polymer based on polyalkylene
25 oxide block copolymer:

40 First, Polyethylene glycol-co-polycaprolactone
polyol ("F68C2") was synthesized as follows:

30 30 g of Pluronic F68 was dried under vacuum at
110 °C for 6 h and then mixed with 1.710 g of
45 caprolactone and 30 mg of stannous 2-ethylhexanoate in a
glass sealing tube. The glass tube then was sealed under
nitrogen atmosphere and heated to 170 °C and maintained
at this temperature for 16 h. The Pluronic F68-
50 caprolactone polymer was cooled and recovered by breaking
35 the glass sealing tube, and then further purified by
several precipitations from a toluene-hexane solvent-

nonsolvent system.

The polymer then was dried in vacuum at 40 °C and used immediately in the activation reaction described below:

5 Reaction with succinic anhydride ("F68C2S"):
 30 g of Pluronic F68-caprolactone copolymer was
 dissolved in 200 ml dry N,N-dimethyl formamide ("DMF")
15 and 0.845 g of succinic anhydride was added to the
 reaction mixture. The mixture was heated to 100 °C under
20 a nitrogen atmosphere for 16 h. The solution then was
 cooled and added to 4000 ml hexane to precipitate the
 carboxyl terminated polymer. It was further purified by
25 repeated (3 times) precipitation from a toluene-hexane
 solvent-nonsolvent system. The polymer was dried under
 vacuum at 40 °C.

25 This polymer was immediately used in activation
 reaction described below:

 Activation of carboxyl groups with N-
30 hydroxysuccinimide ("F68C2SSNHS"):

20 30 g of Pluronic F68-caprolactone succinate
 copolymer was dissolved in 200 ml dry DMF. The solution
 was cooled to 4° C and 1.504 g of 1,3-dicyclohexyl
35 carbodiimide ("DCC") and 1.583 g of N-
 hydroxysulfosuccinimide ("SNHS") were added to the
25 reaction mixture. The mixture was stirred at 4 °C for 6
 h and then stirred overnight at room temperature under
40 nitrogen atmosphere. Dicyclohexylurea was removed by
 filtration and the F68C2S-SNHS derivative was isolated by
 removing the DMF under vacuum and repeated precipitation
30 using a toluene-hexane solvent-nonsolvent system. The
45 product was stored under nitrogen atmosphere at -20 °C.

Example 2. Amine terminated synthetic biodegradable
crosslinkable polymer:

50 35 Reaction of F68TMC2SSNHS with Lysine:
 3.55 g of lysine was dissolved in 200 ml 0.1M

5 borate buffer (pH 8.5). The mixture was cooled to 0 °C in
ice bath and 10 g of F68C2SSNHS were added to the
mixture. The mixture was stirred for 6 h at room
10 temperature and lyophilized. The lyophilized powder was
5 dissolved in 30 ml toluene and filtered. The filtrate
was added to 4000 ml cold diethyl ether. The
precipitated amine terminated polymer was recovered by
15 filtration and dried under vacuum. The polymer was
stored under argon at -20 °C.

10

20 Example 3. Synthesis of carboxyl terminated oligolactic
acid polymer activated with N-hydroxysulfosuccinimide:

Synthesis of difunctional oligolactate with
terminal carboxyl acid end-groups activated with N-
25 hydroxysulfosuccinimide groups.

Part 1: Synthesis of oligomeric poly(lactic
acid) with terminal carboxyl acid groups ("PLA-S"):

In a 250 ml 3 neck flask equipped with
30 mechanical stirrer, nitrogen inlet and distillation
condenser, 2 grams of succinic acid and 34.1 ml 1N HCl
and 3.83 g L-lactic acid, sodium salt were charged. The
flask was then immersed in a silicone oil bath maintained
35 at 150° C. Most of the water from the reaction mixture
was removed over period of 5 hours by distillation. The
25 remaining water was removed by heating the reaction
mixture under vacuum at 180 °C for 15 h. The reaction
mixture was cooled and lyophilized at 0 °C to remove
40 traces of water. The product was isolated by dissolving
in toluene and precipitating in hexane. The precipitated
30 polymer was isolated by filtration and dried in vacuum
for 48 h at 60 °C.

Part 2: Activation of terminal groups with N-
hydroxysulfosuccinimide group:

50 A 3 necked flask equipped with magnetic stirrer
35 and nitrogen inlet was charged with 2 g of PLA-S
copolymer and 20 ml DMF. The solution was cooled 4 °C

5 and 3.657 g of N-hydroxysulfosuccinimide and 3.657 g of
1,3-dicyclohexyl carbodiimide were added to the reaction
mixture. The mixture was stirred at 4 °C for 6 h and
10 overnight at room temperature under nitrogen atmosphere.
5 Dicyclohexylurea was removed by filtration and SNHS
derivative was by isolated by removing the DMF under
vacuum and repeated precipitation using toluene-hexane
15 solvent-nonsolvent system. The product was stored under
nitrogen atmosphere at 4 °C.

10
20 **Example 4.** Preparation of polyethylene glycol based
tetrafunctional crosslinker:

Part 1: Synthesis of tetrafunctional
polyethylene glycol-co-polyglycolate copolymer

15 ("4PEG2KG"):

25 30 grams of 4 arm polyethylene glycol,
molecular weight 2000 ("4PEG2K") was dried at 100 °C for
16 hours prior to use. 30 grams 4PEG2K, 7.66 g of
glycolide and 25 mg of stannous 2-ethylhexanoate were
30 charged into a 3-necked flask equipped with a Teflon
coated magnetic stirring needle. The flask was then
immersed into silicone oil bath maintained at 160 °C.
35 The polymerization reaction was carried out for 16 h
under nitrogen atmosphere. At the end of the reaction,
25 the reaction mixture was dissolved in 100 ml toluene.
The hydroxy terminated glycolate copolymer was isolated
40 by pouring the toluene solution in 4000 ml cold hexane.
It was further purified by repeated dissolution-
precipitation process from toluene-hexane solvent-
30 nonsolvent system and dried under vacuum at 60 °C. It
45 then was immediately used for end capping reaction
mentioned below:

Part 2: Conversion of hydroxyl groups into
carboxylic groups ("4PEG2KGS") and SNHS ester.

50 35 30 g of 4PEG2KG copolymer was dissolved in 150
ml dry pyridine. 8.72 g of succinic anhydride was added

5 to it and the solution was refluxed for 2 h under
nitrogen atmosphere. The polymer was isolated by pouring
10 the cold pyridine solution to 4000 ml hexane. The acid
terminated polymer ("4PEG2KGS") was used in SNHS
5 activation reaction. Briefly, to a solution of 30 g of
4PEG2KGS in 300 ml dry methylene chloride were added
10.58 g of SNHS and 10.05 g DCC. The reaction mixture
15 was stirred overnight under nitrogen atmosphere.
Dicyclohexylurea was removed by filtration. The filtrate
10 was evaporated and the residue obtained was redissolved
in 100 ml toluene. The toluene solution was precipitated
20 in 2000 ml hexane. The SNHS activated polymer was stored
under nitrogen atmosphere until further use.

25 **Example 5. Sulfonyl chloride activated crosslinkers:**

Activation of tetrafunctional polyethylene
glycol-co-polyglycolate copolymer ("4PEG2KGS") with
tresyl chloride:

30 30 g of 4PEG2KG was dissolved in 10 ml dry
20 benzene. The solution was cooled to 0°C and 5.92 g of
triethyl amine and 10.70 g tresyl chloride were added
under nitrogen atmosphere. After refluxing for 3h under
35 nitrogen atmosphere, the reaction mixture was cooled and
filtered to remove triethylamine hydrochloride. The
25 filtrate was poured into 3000 ml hexane to precipitate
the activated polymer. The residue was redissolved in
40 THF and filtered over neutral alumina to remove traces of
triethylamine hydrochloride. The polymer was recovered
by adding the THF solution to 3000 ml diethyl ether and
30 stored under nitrogen atmosphere.

45 **Example 6. Synthesis of multifunctional
oligopolycaprolactone terminated with SNHS:**

Part 1: Synthesis of polycaprolactone ("PCL1"):

50 35 2.00 g of glycerol, 8.17 g of caprolactone and
50 mg of stannous 2-ethylhexanoate were charged into 100

5 ml Pyrex pressure sealing tube. The tube was frozen in
liquid nitrogen and connected to vacuum line for 10
10 minutes. The tube then was connected to argon gas line
and sealed under argon. The sealed reaction mixture then
5 was immersed in oil bath maintained at 160°C and
polymerization was carried out for 16 h at 160°C. The
15 polymer was recovered by dissolving it in 30 ml toluene
and precipitating in 2000 ml cold hexane. The
precipitated liquid oligomer was recovered and dried
10 under vacuum for 1 day at 60°C.

20 Part 2: End-capping of PCL1 with succinic
anhydride ("PCL-S"):

10 g of PCL1 was dissolved in 150 ml dry
benzene. About 50 ml of benzene was distilled to remove
25 traces of water from the reaction mixture. The solution
was cooled to 30°C. To this warm solution, 6.67 g of
triethyl amine and 7.86 g of succinic anhydride were
added. The reaction mixture was then refluxed for 6 h
30 and concentrated by distillation under vacuum. The
20 product was recovered by adding the filtrate to 2000 ml
cold dry hexane.

Part 3: Activation of PCL-S with SNHS:

35 PCL1-succinate (5.0 g) was dissolved in 10 ml
of anhydrous methylene chloride, cooled to 0°C and 7.82 g
25 of N-hydroxysulfosuccinimide and 7.42 N, N-
dicyclohexylcarbodiimide were added under stirring.
40 After stirring the mixture overnight, the precipitated
dicyclohexylurea was removed by filtration and the
solution was concentrated by removing solvent. The ¹H-NMR
30 spectrum showed succinimide singlet at 2.80 ppm (2H).

45
50 **Example 7.** Preparation of polyethylene glycol-co-
polytrimethylene carbonate copolymer terminated with N-
hydroxysuccinimide:

35 Preparation of tetrafunctional polyethylene
glycol-co-polytrimethylene carbonate copolymer

5 ("4PEG10KTCM2"):

30 g of tetrahydroxy polyethylene glycol,
molecular weight 10000, was dried under vacuum at 90-
10 100°C in a glass sealing tube. The tube then was cooled
5 and transferred inside an air bag where 2.45 g of
trimethylene carbonate and 20 mg of stannous octoate were
15 added to the tube. The glass tube was then sealed under
vacuum and heated with stirring at 155°C and maintained
at this temperature for 16 h. The polyethylene glycol-
20 co-polytrimethylene carbonate polymer was cooled and
recovered by breaking the glass sealing tube. It was
25 further purified by several precipitations from toluene-
hexane solvent-nonsolvent system.

Part 2: Synthesis of glutarate derivative of
15 4PEG10KTCM2 ("4PEG10KTCM2G"):

10 g of 4PEG10KTCM2 was dissolved in 120 ml dry
toluene. About 50 ml of toluene was distilled to remove
traces of water from the reaction mixture. The warm
30 solution was cooled to 60°C. To this solution, 1.23 g of
20 triethyl amine and 1.40 g of glutaric anhydride were
added. The reaction mixture was heated to 60°C for 1 h
and filtered. The product was recovered by adding the
35 filtrate to 2000 ml cold dry hexane.

Part 3: Activation of terminal carboxyl groups
25 using N-hydroxysuccinimide ("4PEG10KTCM2GNHS"):

30 g of 4PEG10KTCM2G was dissolved in 100 ml of
40 dry DMF and 1.53 g of N-hydroxysuccinimide and 5 g
molecular sieves 3A° were added. 1.28 g of DCC dissolved
in 5 ml dry DMF was added dropwise and the reaction
30 mixture was kept at room temperature for 24 h under
45 nitrogen atmosphere. The mixture was diluted with 50 ml
cold benzene and precipitated using cold hexane. The
precipitate was collected on a sintered glass filter with
50 suction. The dissolution and precipitation procedure was
35 then repeated three times, using toluene-diethyl ether as
solvent-nonsolvent system and dried under vacuum. The

product was stored under nitrogen atmosphere at -20°C until further use.

Example 8. Succinated polyhydroxy compounds activated with N-hydroxysulfosuccinimide ES:

10 g of erythritol was dissolved in 200 ml dry toluene. About 50 ml of toluene was distilled to remove traces of water from the erythritol. The solution was cooled to 50-60°C and 20 ml pyridine and 8.58 g of succinic anhydride were added to the solution. The reaction mixture was then refluxed for 3 h and unreacted pyridine and toluene were evaporated to dryness under reduced pressure. The residue was used in activation reaction.

Part 2: Activation of ES with SNHS:

Erythritol-succinate (ES, 2.0 g) was dissolved in 10 ml of anhydrous dimethyl formamide ("DMF"), cooled to 0°C and 3.47 g of N-hydroxysulfosuccinimide and 3.30 N, N-dicyclohexylcarbodiimide were added under stirring. After stirring the mixture overnight, the precipitated dicyclohexylurea was removed by filtration and the solution was concentrated by removing solvent. It was further purified by column chromatography.

Example 9. Preparation of synthetic crosslinked biodegradable gels:

1.57 g (0.8 mM) of 4 arm amine terminated polyethylene glycol molecular weight 2000 was dissolved in 10 ml 0.1 M sodium borate buffer at pH 9.5. 2 g of 4 arm SNHS activated 4PEG2KGS polymer (molecular weight 2500) was dissolved in phosphate buffered saline. These two solutions were mixed to produce a crosslinked gel. In another variation of this method, the 4PEG2KGS polymer solid was directly added to the amine terminated polymer solution to produce a crosslinked polymer.

5 In another variation, a crosslinker consisting
of an equimolar solution of dilysine can be used in place
10 of the 4 arm PEG amine solution to form a hydrogel.
Gelation was seen to occur within 10 seconds of mixing
5 the two solutions. Similarly, other crosslinkers
described in examples 1 to 7 may be reacted in molar
equivalent proportions with other amine terminated
15 polymers such as albumin or amine terminated
biodegradable polymers similar to described in Example 2.
20 The preferred compositions for making biodegradable
hydrogels were described in Table 2. The amine
terminated polymer solution described above was added
with 0.1% of F D and C blue or indigo dye prior to
crosslinking reaction. The addition of dye allows the
25 preparation of colored gels.

**Example 10. Preparation of composite synthetic
crosslinked colored biodegradable gels:**

30 3 grams of bovine serum albumin was dissolved
20 in 3 ml of phosphate buffered solution. Commercial
sutures based on synthetic biodegradable polymers, such
as Vicryl was cut/ground into several small pieces (size
35 less than 1 mm) using cryogenic grinding. These colored
suture particles (approximately 100 mg) were mixed with
25 the albumin solution to form a suspension. 100 mg of
crosslinker such as 4PEG10KTC2GNHS was mixed with 0.2 ml
40 of albumin suspension. This viscous solution then was
mixed with 40 mg of triethanol amine (buffering agent).
The addition of triethanol amine gels the solution in 60
30 seconds. The colored suture particles entrapped in the
45 crosslinked gel help to visualize the gel especially when
under laparoscopic conditions and also acts to strengthen
the hydrogel as a reinforcing agent. The suture
particles in above examples can be replaced with
50 biodegradable microparticles loaded with drugs or
bioactive compounds.

5 Example 11. Formulation of SG-PEG with Di-lysine:

 A four arm PEG with SG end groups (Shearwater
Polymers, approx. 9,100 g/mol, 0.704 grams, 6.5×10^{-5}
10 moles) was dissolved in 2.96 g 0.01M pH 4.0 phosphate
5 buffer (19.2% solids). Di-lysine (Sigma, 347.3 g/mol,
0.03 grams, 8.7×10^{-5} moles) was dissolved in 3.64 grams of
15 0.1M pH 9.5 borate buffer (0.8% solids). On combination
of the two solutions, the percent solids was 10%. The di-
lysine has 3 amine groups. The SG-PEG has 4 NHS groups.
20 After correction for the less than 100% degree of
substitution on the SG-PEG, the formulation gives a 1:1
stoichiometry of amine groups to NHS groups.

 Example 12. Formulation of SG-PEG with Tri-lysine:

25 A four arm PEG with SG end groups (Shearwater
Polymers, approx. 9,100 g/mol, 0.675 grams, 6.2×10^{-5}
moles) was dissolved in 2.82 g 0.01M pH 4.0 phosphate
30 buffer (19.3% solids). Tri-lysine (Sigma, 402.5 g/mol,
0.025 grams, 6.2×10^{-5} moles) was dissolved in 3.47 grams
20 of 0.1M pH 9.5 borate buffer (0.7% solids). On
combination of the two solutions, the percent solids was
10%. The tri-lysine has 4 amine groups. The SG-PEG has
35 4 NHS groups. After correction for the less than 100%
degree of substitution on the SG-PEG, the formulation
25 gives a 1:1 stoichiometry of amine groups to NHS groups.

40 Example 13. Formulation of SG-PEG with Tetra-lysine:

 A four arm PEG with SG end groups (Shearwater
Polymers, approx. 9,100 g/mol, 0.640 grams, 5.9×10^{-5}
30 moles) was dissolved in 2.68 g 0.01M pH 4.0 phosphate
45 buffer (19.2% solids). Tetra-lysine (Sigma, 530.7 g/mol,
0.025 grams, 4.7×10^{-5} moles) was dissolved in 3.30 grams
of 0.1M pH 9.5 borate buffer (0.8% solids). On
50 combination of the two solutions, the percent solids was
35 10%. The tetra-lysine has 5 amine groups. The SG-PEG
has 4 NHS groups. After correction for the less than

100% degree of substitution on the SG-PEG, the formulation gives a 1:1 stoichiometry of amine groups to NHS groups.

5 Example 14. Gel Time Measurement:

The amine solution (100 μ L) was aliquotted into a 100x13 test tube. A flea-stirbar (7x2 mm, Fisher Scientific p/n 58948-976) was placed in the test tube. The test tube was held stationary over a digital magnetic stirrer (VWR Series 400S Stirrer) set at 300 rpm. A 1 cc tuberculin syringe (Becton Dickinson, p/n BD309602) was filled with 100 μ L of the ester solution. The syringe was inserted up to the flanges so that the distal end was just over the amine solution. Simultaneously the plunger was depressed and a stop watch started. When the solution solidifies sufficiently so that the stir bar stops spinning, the stop watch was stopped. Each solution was measured in triplicate and the mean \pm 1 standard deviation was plotted. Results for the formulations of examples 1, 2 and 3 are shown in FIG. 11.

Example 15. Change in gel time as a function of ester solution age:

An important characteristic of these systems is the loss in reactivity over time from reconstitution of the ester solution. This loss in reactivity occurs due to hydrolysis of the N-hydroxysuccinimidyl ester, before the activated molecule can combine with its respective nucleophile. The loss of reactivity was characterized by measuring the change in gel time as a function of time from reconstitution of the NHS ester solution. The gel time was measured at $\frac{1}{2}$ hour intervals. The NHS ester solution was stored at ambient conditions during this measurement. Results for the solutions described in Examples 11, 12 and 13 are shown in FIG. 12.

Example 16. Gel formation at different percent solids from 4 arm CM-HBA-NS PEG and Lys-Lys:

Using the gel time method described in Example 13, five different gel compositions were made using carboxymethyl hydroxybutyrate-hydroxysuccinimide end-capped 4 arm PEG (CM-HBA) (Shearwater Polymers) and di-lysine (Sigma). The formulations are listed below in Table 3.

Table 3

Conc. (%)	CM-HBA (g)	Phosphate (g)	Lys-Lys (g)	Borate (g)
8.5	0.2469	1.264	0.01	1.5012
10	0.2904	1.2209	0.012	1.4994
12.5	0.363	1.1483	0.015	1.4964
15	0.4356	1.0757	0.018	1.4936
20	0.5808	0.9305	0.024	1.4876

The formulations were adjusted to give a 1 to 1 ratio of electrophilic end groups on the CM-HBA (4) to nucleophilic reactive groups on the di-lysine ("Lys-Lys") (3). The CM-HBA quantities were dissolved in 0.01M pH 5.0 phosphate buffer. The di-lysine was dissolved in 0.1M pH 11 borate buffer. Gel time results are shown in Figure 13. This data also shows that the higher percent solids solutions also are the most stable with respect to retention of speed of reaction.

Example 17. Degradation of Hydrogels:

Hydrogel plugs made during the gel time measurements of Example 14 were placed in approximately 25 mL 0.1M phosphate buffered saline at pH 7.4 in 50 mL Falcon tubes and placed in a constant temperature bath at 37°C. The hydrogel plugs were observed visually at periodic intervals and the time of gel disappearance noted. The data are plotted in Figure 14.

5 **Example 18.** Precursor Spray Procedure to form a 7.5% solids hydrogel from 4 arm SG and dilysine:

10 An ethylene oxide sterilized air assisted sprayer was used in conjunction with aqueous solutions of
5 polymerizable monomers. Solution 1 consisted of a 14.4% solution of 4 arm SG (MW 10,000 Da, purchased from
15 Shearwater Polymers) dissolved in 0.01M phosphate buffer at pH 4.0 and was sterile filtered (Pall Gelman syringe filter, p/n 4905) and drawn up in a sterile 5 cc syringe.
20 Solution 2 consisted of a 1.2% solution of a dilysine (purchased from Sigma Chemicals) dissolved in 0.1M borate buffer at pH 11 with 0.5 mg/mL methylene blue for visualization and was also sterile filtered and drawn up in a sterile 5 cc syringe. These solutions, when
25 combined 1:1 on a volumetric basis, result in a 1:1 ratio of NHS ester to amine end group. The final % solids after combination is 7.5%. The two syringes were individually loaded in the two separate receptacles through a luer-lok type of linkage. Airflow from a
30 regulated source of compressed air (an air compressor such as those commercially available for airbrushes) was connected to the device using a piece of Tygon tube.
35 On compressing the syringe plungers a steady spray of the two liquid components was observed. When this spray was
40 directed to a piece of tissue (rat cecum) a hydrogel coating was observed to form on the surface of the tissue. This hydrogel coating was rinsed with saline (the hydrogel coating is resistant to rinsing) and was observed to be well adherent to the tissue surface.
45 Within a short period of time (less than a minute) an area of 10 cm X 5 cm could be coated with ease.

50 **Example 19.** Precursor Spray Procedure to form a 12.5% solids hydrogel from 4 arm CM and dilysine:

55 A hydrogel barrier film made from 4 arm CM-HBA NS (MW 10,000 Da, purchased from Shearwater Polymers),

5 and dilysine was similarly prepared and sprayed as
described in Example 18. In the present example the 4
10 arm CM solution was made up to 24.0% solids and the
dilysine solution was made up to 1.0% solids such that on
5 combination in an equal volume delivery system a 1:1
ratio of NHS to amine end groups results, giving a final
15 %solids of 12.5%.

Example 20. Spray Application of crosslinker and polymer
10 to from crosslinked film:

20 Two solutions (component A and component B)
were prepared. Component A consisted of dilysine in 0.1M
borate buffer, pH 9.5. Component B consisted of either 4
arm SG-PEG or 4 arm CM-HBA-NS in 0.01M phosphate buffer,
25 pH 4.0. These solutions were prepared such that the
amine to ester stoichiometric ratio was 1:1 and the final
total solution concentration was 7.5% or 12.5%,
respectively.

30 A Fibriject™ (Micromedics, Inc) 5 cc syringe
20 holder and cap was used, preloaded with 5 cc of each
solution and attached to a dual barrel atomizing sprayer.
The sprayer has two hubs for the syringes to connect to
35 allowing the two fluids to be advanced through two
separate lumens over any preset distance. A third hub
25 exists for the application of the atomizing gas. Air was
used in this example. The distal tip of the sprayer
contains a chamber where the gas expands out of an
40 introduction tube, then flows past the two polymer
solution nozzles in an annular space around each. The
30 gas is accelerated in the annular spaces using a flow
45 rate suitable for the complete atomization of the two
fluid streams (~2L/min.). Two overlapping spray cones
are thus formed allowing for well mixed, thin, uniform
50 coatings to be applied to surfaces.

35

Example 21. Adhesion Prevention in Rat Cecum Model:

Surgical procedure:

Male Sprague Dawley rats (250-350 grams,) were anesthetized with an intramuscular 4ml/kg "cocktail" of Ketamine (25 mg/ml), Xylazine (1.3mg/mL) and Acepromazine (0.33 mg/mL). The abdominal area was shaved and prepped for aseptic surgery. A midline incision was made to expose the abdominal contents. The cecum was identified and location within the abdomen was noted. The cecum was pulled out of the abdomen and the surface of one side was abraded using dry sterile gauze. A technique of abrading one area by stroking the surface 12 times with the gauze was used. The cecal arterial supply was interrupted using bipolar coagulation along the entire surface area of the damaged cecum.

The opposing abdominal sidewall which lays in proximity to the damaged cecal surface was deperitonealized with a scalpel blade and the underlying muscle layer was scraped to the point of hemorrhaging.

The cecum was sprayed with either the SG-PEG system or the CM-HBA-NS system using the air assisted spray method described in the preceding example. The cecum was placed with the damaged (ischemic area) side up opposite the damaged side wall. Active bleeding was controlled before closing. The peritoneum and muscle wall was closed with 3-0 nylon and the skin was closed with 4-0 silk. Rats were returned to their cages for one to two weeks at which time evaluation of the adhesion between the side wall and cecum was noted. The rats were killed at 10 days and the tenacity and extent of adhesion was evaluated. The results are summarized in Table 4.

Table 4

Rat #	Material Applied	Reference Example	Findings on Day 10
403	7.5% 4aSG with Lys-Lys w/MB	Example 18	Small amount of gel present on cecum. No adhesions from cecum to sidewall. No gel on sidewall.
404	7.5% 4aSG with Lys-Lys w/MB	Example 18	Some mesentary stuck to cecum. No gel. No adhesions.
405	7.5% 4aSG with Lys-Lys w/MB	Example 18	Small amount of gel present on cecum. Some mesentary stuck to cecum and sidewall. Some gel between mesentary and cecum where stuck. No adhesions.
406	12.5% 4aCM with Lys-Lys w/MB	Example 19	No gel present. No adhesions.
407	12.5% 4aCM with Lys-Lys w/MB	Example 19	No gel on cecum or sidewall. No adhesions.
408	12.5% 4aCM with Lys-Lys w/MB	Example 19	Rat died post-op (anesthesia overdose).

* * *

While preferred illustrative embodiments of the invention are described above, it will be apparent to one skilled in the art that various changes and modifications may be made therein without departing from the invention, and it is intended in the appended claims to cover all such changes and modifications which fall within the true spirit and scope of the invention.

Claims

5

10

15

20

25

30

35

40

45

50

55

What is claimed is:

1. A method for preparing a biocompatible crosslinked polymer, comprising:

providing a biocompatible small molecule crosslinker having n crosslinker functional groups, wherein n is two or more, and wherein the crosslinker functional groups are either electrophilic or nucleophilic;

dissolving the biocompatible small molecule crosslinker in a first solvent to form a crosslinker solution;

providing a biocompatible functional polymer having m functional polymer functional groups, wherein m is two or more and the sum of n and m is five or more, and wherein the functional polymer functional groups are nucleophilic if the crosslinker functional groups are electrophilic, and the functional polymer functional groups are electrophilic if the crosslinker functional groups are nucleophilic;

dissolving the biocompatible functional polymer in a second solvent to form a functional polymer solution; and

combining the crosslinker and functional polymer solutions to react the crosslinker functional groups with the functional polymer functional groups.

2. The method of claim 1, wherein combining the crosslinker and functional polymer solutions further comprises combining the crosslinker and functional polymer solutions in an animal or human body.

3. The method of claim 1, wherein providing a biocompatible small molecule crosslinker further comprises providing a biocompatible small molecule crosslinker having a solubility of at least 1 g/100 ml in an aqueous solution.

5
10
15
20
25
30
35
40
45
50
55

4. The method of claim 1, wherein providing a biocompatible small molecule crosslinker further comprises providing a biocompatible small molecule crosslinker having crosslinker functional groups that are electrophilic.

5. The method of claim 4, wherein providing a biocompatible small molecule crosslinker having crosslinker functional groups that are electrophilic further comprises providing a biocompatible small molecule crosslinker wherein the electrophilic crosslinker functional groups are N-hydroxysuccinimide groups.

6. The method of claim 5, wherein providing a biocompatible functional polymer further comprises providing a biocompatible functional polymer wherein the functional polymer functional groups are amines.

7. The method of claim 1, wherein providing a biocompatible small molecule crosslinker further comprises providing a biocompatible small molecule crosslinker having crosslinker functional groups that are nucleophilic.

8. The method of claim 7, wherein providing a biocompatible small molecule crosslinker having crosslinker functional groups that are nucleophilic further comprises providing a biocompatible small molecule crosslinker wherein the crosslinker functional groups are amines.

9. The method of claim 8, wherein providing a biocompatible functional polymer further comprises providing a biocompatible functional polymer wherein the

functional polymer functional groups are N-hydroxysuccinimide groups.

10. The method of claim 1, wherein providing a biocompatible small molecule crosslinker further comprises providing a biocompatible small molecule crosslinker having a biodegradable link.

11. The method of claim 1, wherein providing a biocompatible functional polymer further comprises providing a biocompatible functional polymer having a biodegradable link.

12. The method of claim 1, wherein combining the crosslinker and functional polymer solutions further comprises reacting the crosslinker functional groups and the functional polymer functional groups to produce a biodegradable link.

13. The method of claim 1, further comprising: providing a visualization agent; and dissolving the visualization agent in the first solvent.

14. The method of claim 1, further comprising: providing a visualization agent; and dissolving the visualization agent in the second solvent.

15. A biocompatible small molecule crosslinker having n functional groups, wherein n is two or more.

16. The biocompatible small molecule crosslinker of claim 15, wherein the biocompatible small molecule crosslinker has a solubility of at least 1 g/100 ml in an aqueous solution.

5
10 17. The biocompatible small molecule crosslinker of claim 15, wherein the functional groups are nucleophilic.

15 18. The biocompatible small molecule crosslinker of claim 17, wherein the functional groups are amines.

20 19. The biocompatible small molecule crosslinker of claim 15, wherein the biocompatible small molecule crosslinker further comprises a biodegradable link.

25 20. A biocompatible crosslinked polymer, comprising:
at least one biocompatible small molecule crosslinker regions;
at least one biocompatible functional polymer regions,
30 wherein the biocompatible crosslinked polymer comprises at least three links between the crosslinker regions and the functional polymer regions, and the links
35 are a reaction product of electrophilic functional groups with nucleophilic functional groups.

40 21. The biocompatible crosslinked polymer of claim 20, wherein the biocompatible small molecule crosslinker regions each have a solubility of at least 1 g/100 ml in an aqueous solution.

45 22. The biocompatible crosslinked polymer of claim 20, wherein the biocompatible crosslinked polymer further comprises at least one biodegradable link.

50 23. The biocompatible crosslinked polymer of claim 20, wherein at least one of the links between the
55

crosslinker and functional polymer regions is biodegradable.

24. A method for preventing surgical adhesions, the method comprising:

providing, at a surgical site, a first solution comprising a biocompatible small molecule crosslinker having n crosslinker functional groups, wherein n is two or more, and wherein the crosslinker functional groups are either electrophilic or nucleophilic;

providing, at the surgical site, a second solution comprising a biocompatible functional polymer having m functional polymer functional groups, wherein m is two or more and the sum of n and m is five or more, and wherein the functional polymer functional groups are nucleophilic if the crosslinker functional groups are electrophilic, and the functional polymer functional groups are electrophilic if the crosslinker functional groups are nucleophilic; and

combining the first and second solutions to react the crosslinker functional groups with the functional polymer functional groups and produce a biocompatible crosslinked polymer at the surgical site.

25. The method of claim 24, wherein providing a first solution further comprises providing a first solution comprising a biocompatible small molecule crosslinker having a solubility of at least 1 g/100 ml in an aqueous solution.

26. A method for drug delivery comprising:

providing a first solution comprising a biocompatible small molecule crosslinker having n crosslinker functional groups, wherein n is two or more, and wherein the crosslinker functional groups are either electrophilic or nucleophilic;

5 providing a second solution comprising a
biocompatible functional polymer having m functional
10 polymer functional groups, wherein m is two or more and
the sum of n and m is five or more, and wherein the
functional polymer functional groups are nucleophilic if
the crosslinker functional groups are electrophilic, and
15 the functional polymer functional groups are
electrophilic if the crosslinker functional groups are
nucleophilic;

providing a drug;

20 combining the first and second solutions and
the drug to react the crosslinker functional groups with
the functional polymer functional groups and produce a
biocompatible crosslinked polymer entrapping the drug;
25 and

injecting or implanting the biocompatible
crosslinked polymer in an animal or human body.

30 27. The method of claim 26, wherein providing
a first solution further comprises providing a first
solution comprising a biocompatible small molecule
crosslinker having a solubility of at least 1 g/100 ml in
35 an aqueous solution.

28. A method for drug delivery comprising:

40 providing, in an animal or human body, a first
solution comprising a biocompatible small molecule
crosslinker having n crosslinker functional groups,
wherein n is two or more, and wherein the crosslinker
functional groups are either electrophilic or
45 nucleophilic;

providing, in the body, a second solution
comprising a biocompatible functional polymer having m
50 functional polymer functional groups, wherein m is two or
more and the sum of n and m is five or more, and wherein
the functional polymer functional groups are nucleophilic

5
if the crosslinker functional groups are electrophilic,
and the functional polymer functional groups are
electrophilic if the crosslinker functional groups are
10 nucleophilic;

providing, in the body, a drug; and
combining, in the body, the first and second
15 solutions and the drug to react the crosslinker
functional groups with the functional polymer functional
groups and form a biocompatible crosslinked polymer
entrapping the drug.

20
29. The method of claim 28, wherein providing
a first solution further comprises providing a first
solution comprising a biocompatible small molecule
25 crosslinker having a solubility of at least 1 g/100 ml in
an aqueous solution.

30. A method for completely or partially
30 blocking, augmenting, sealing or filling a natural or
surgically-created void, lumen or space in an animal or
human body, the method comprising:

providing a first solution comprising a
35 biocompatible small molecule crosslinker having n
crosslinker functional groups, wherein n is two or more,
and wherein the crosslinker functional groups are either
electrophilic or nucleophilic;

40 providing a second solution comprising a
biocompatible functional polymer having m functional
polymer functional groups, wherein m is two or more and
the sum of n and m is five or more, and wherein the
45 functional polymer functional groups are nucleophilic if
the crosslinker functional groups are electrophilic, and
the functional polymer functional groups are
electrophilic if the crosslinker functional groups are
50 nucleophilic;

combining the first and second solutions

5 solutions to react the crosslinker functional groups with
the functional polymer functional groups and produce a
biocompatible crosslinked polymer; and

10 injecting or implanting the biocompatible
crosslinked polymer in the void, lumen or space.

15 31. The method of claim 30, wherein providing
a first solution further comprises providing a first
solution comprising a biocompatible small molecule
crosslinker having a solubility of at least 1 g/100 ml in
20 an aqueous solution.

25 32. A method for completely or partially
blocking, augmenting, sealing or filling a natural or
surgically-created void, lumen or space in an animal or
human body, the method comprising:

30 providing, in the void, lumen or space, a first
solution comprising a biocompatible small molecule
crosslinker having n crosslinker functional groups,
wherein n is two or more, and wherein the crosslinker
functional groups are either electrophilic or
nucleophilic;

35 providing, in the void, lumen or space, a
second solution comprising a biocompatible functional
polymer having m functional polymer functional groups,
wherein m is two or more and the sum of n and m is five
40 or more, and wherein the functional polymer functional
groups are nucleophilic if the crosslinker functional
groups are electrophilic, and the functional polymer
functional groups are electrophilic if the crosslinker
45 functional groups are nucleophilic; and

50 combining the first and second solutions to
react the crosslinker functional groups with the
functional polymer functional groups and produce a
biocompatible crosslinked polymer in the void, lumen or
space.

5
33. The method of claim 32, wherein providing
a first solution further comprises providing a first
10 solution comprising a biocompatible small molecule
crosslinker having a solubility of at least 1 g/100 ml in
an aqueous solution.
15
20
25
30
35
40
45
50
55

1/10

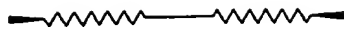


FIG. 1A

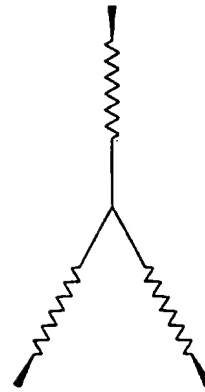


FIG. 1B

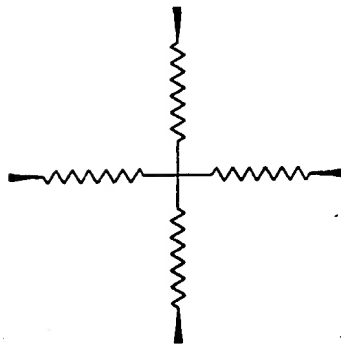


FIG. 1C

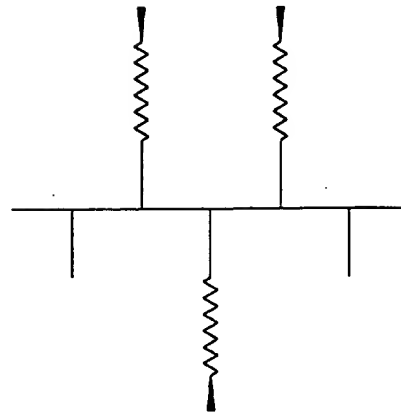


FIG. 1E

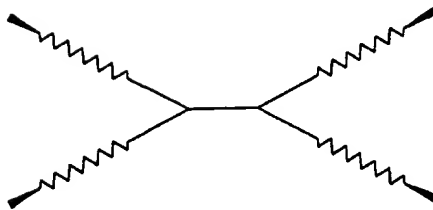


FIG. 1D



FIG. 2F

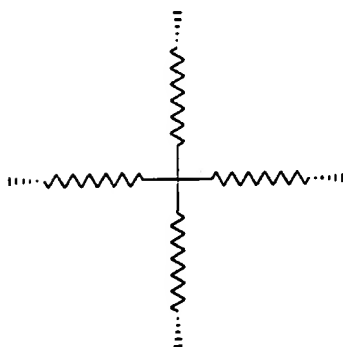


FIG. 2H

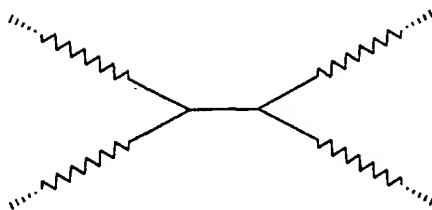


FIG. 2I



FIG. 3K

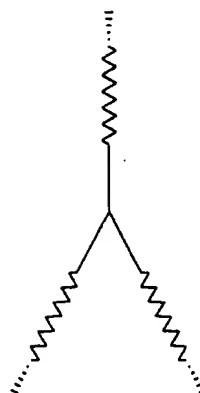


FIG. 2G

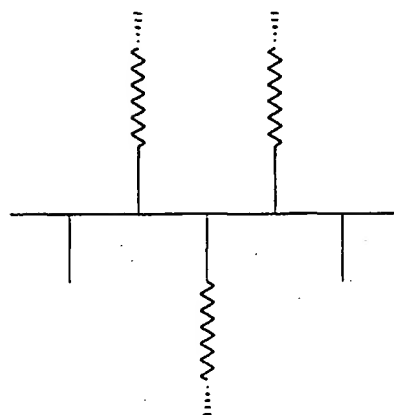


FIG. 2J

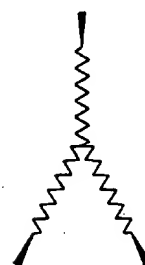


FIG. 3L

3/10

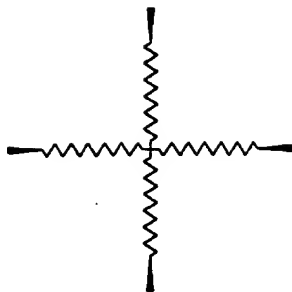


FIG. 3M

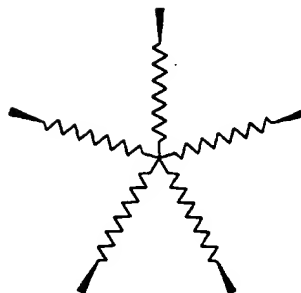


FIG. 3O

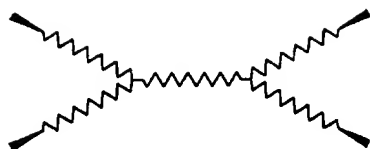


FIG. 3N



FIG. 4P

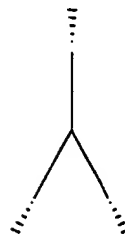


FIG. 4Q

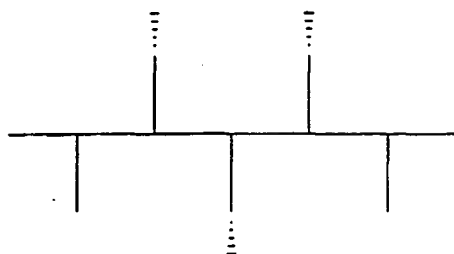


FIG. 4T

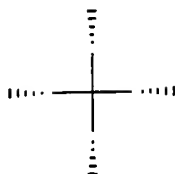


FIG. 4R

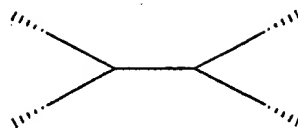


FIG. 4S



FIG. 5U

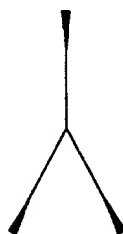


FIG. 5V

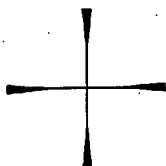


FIG. 5W

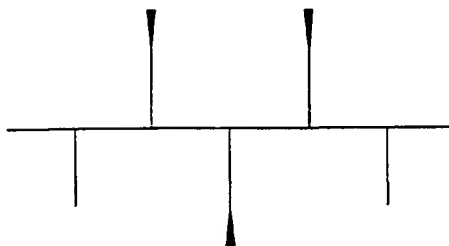


FIG. 5Y

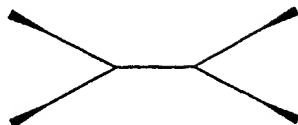


FIG. 5X

5/10

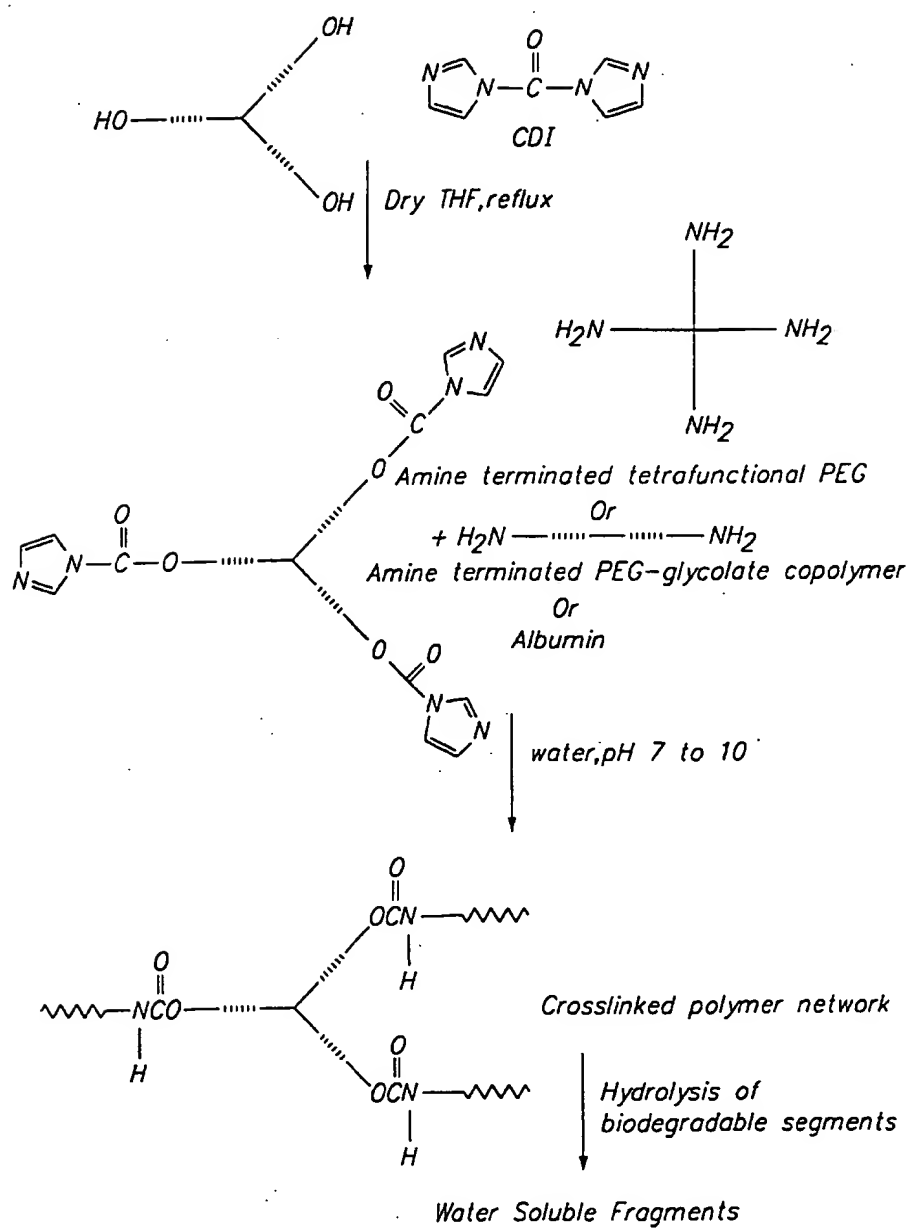



FIG. 6


Hydroxy terminated biodegradable multifunctional polymer

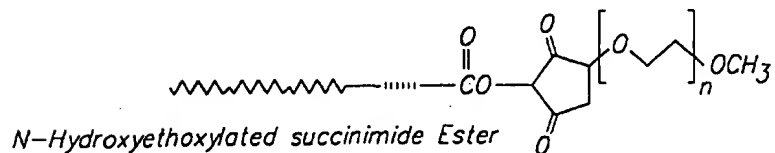
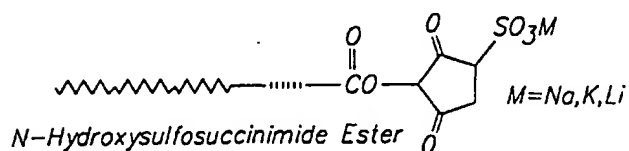
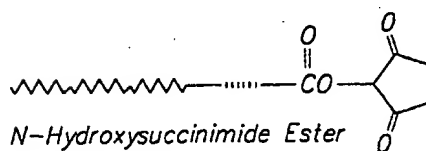
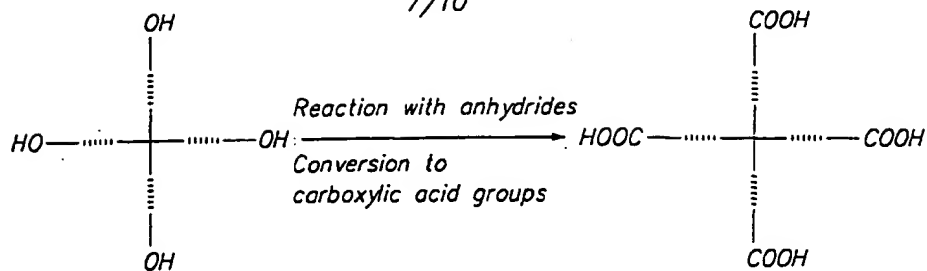


FIG. 9

7/10



Hydroxy terminated 4 arm polymer
like PEO-caprolactone

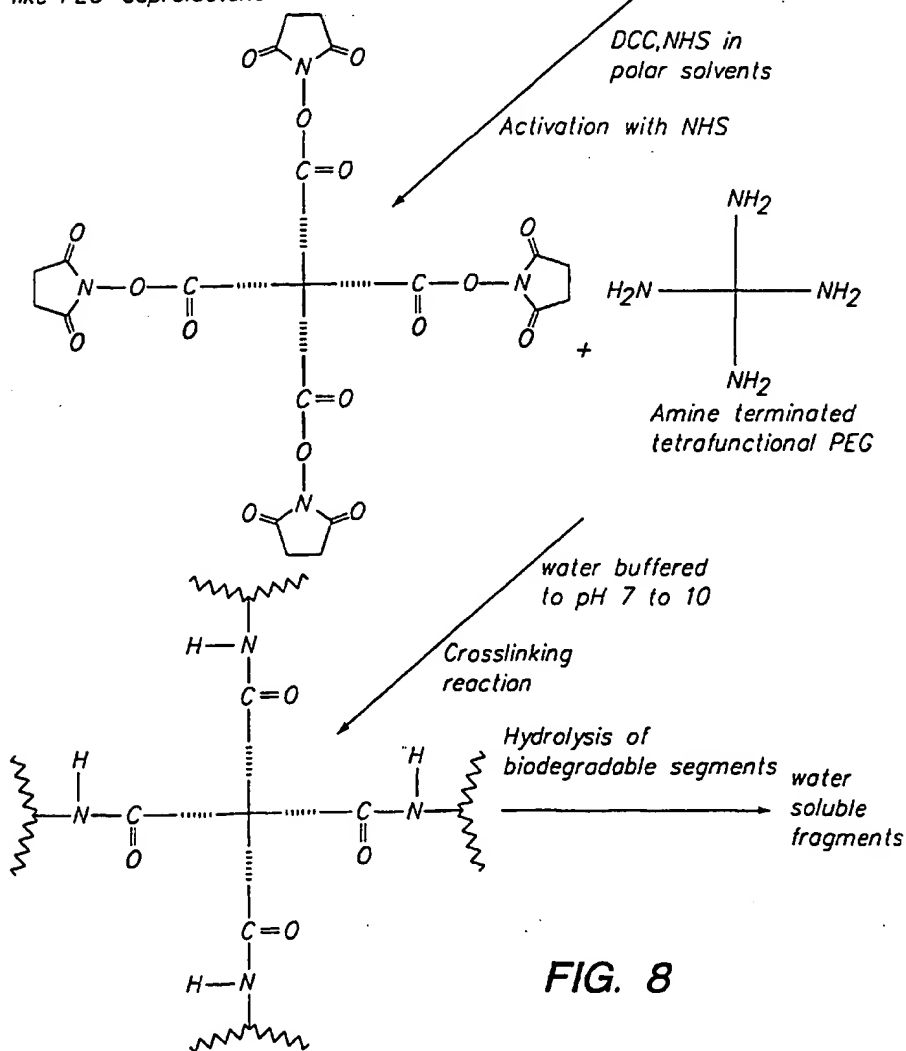


FIG. 8

8/10

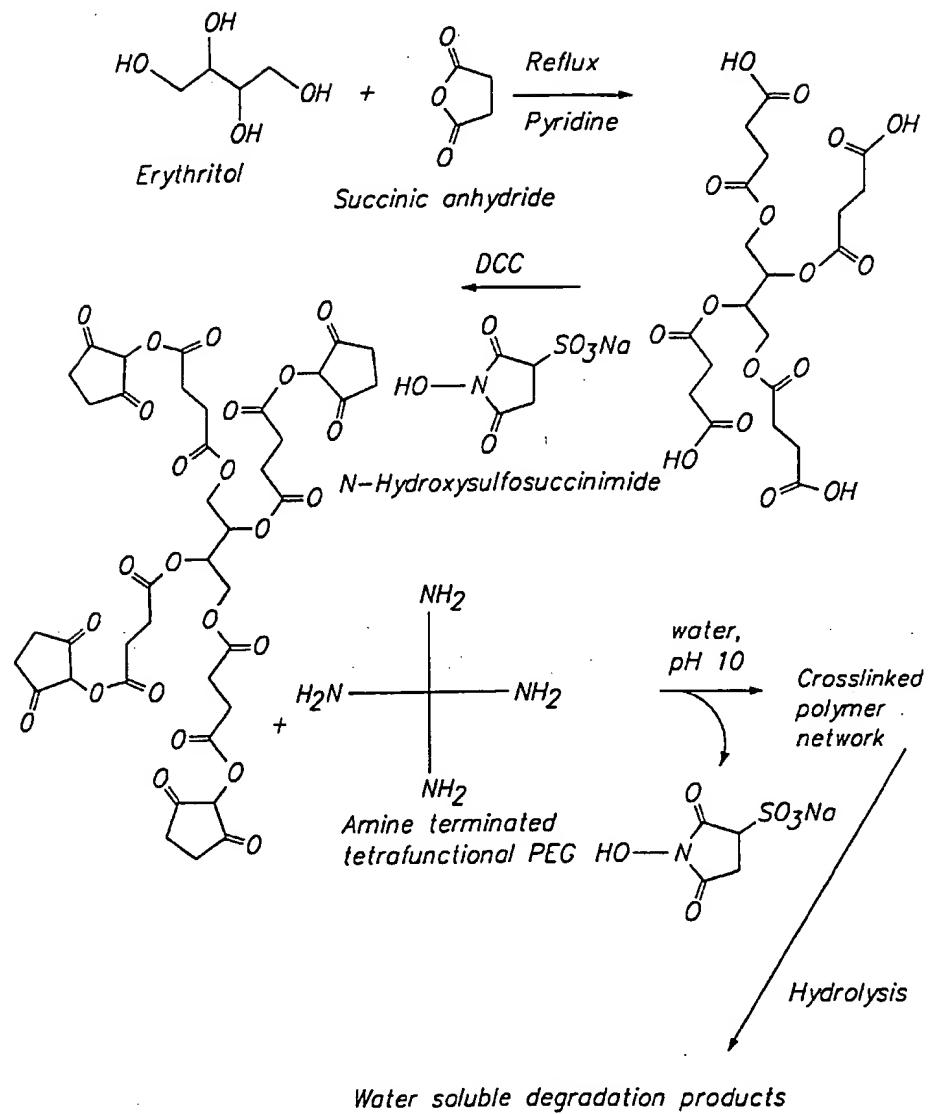


FIG. 10

9/10

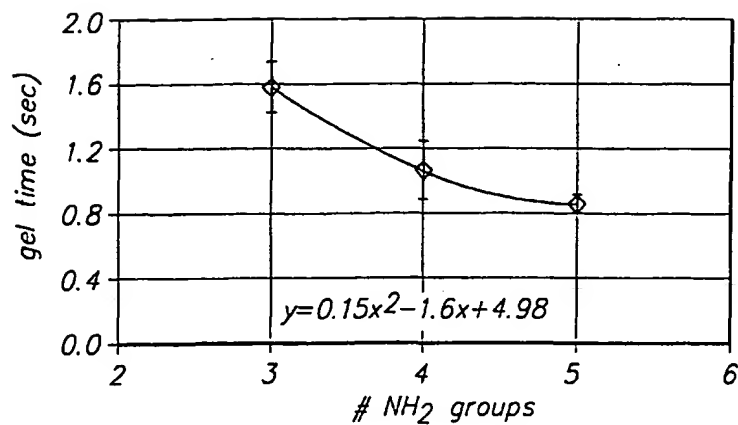


FIG. 11

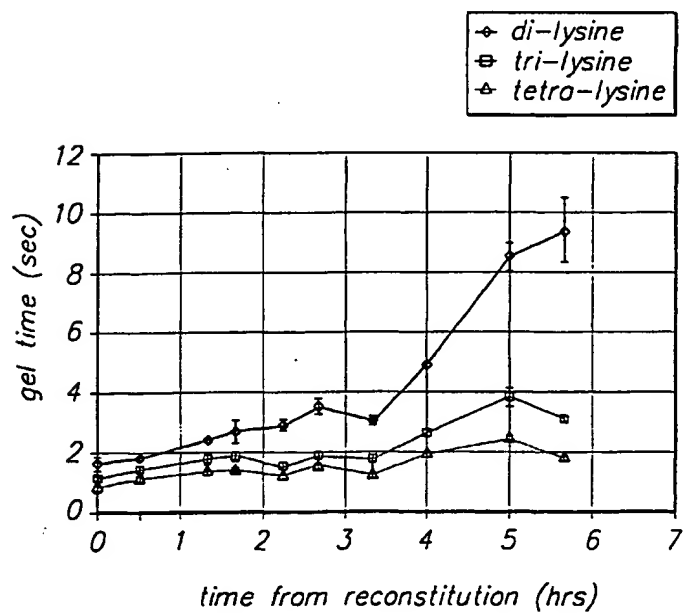


FIG. 12

10/10

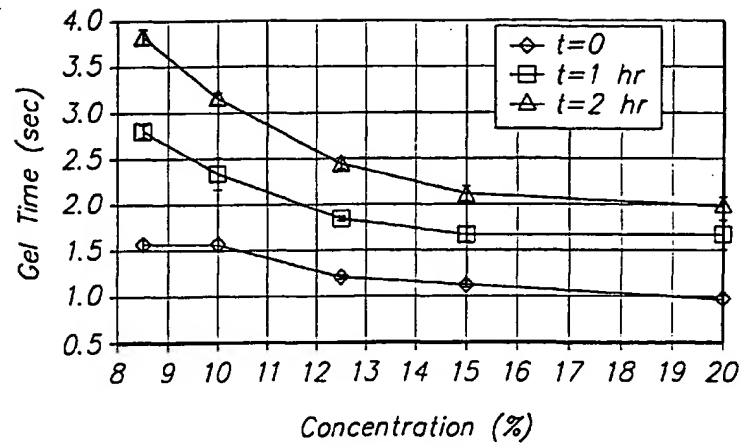


FIG. 13

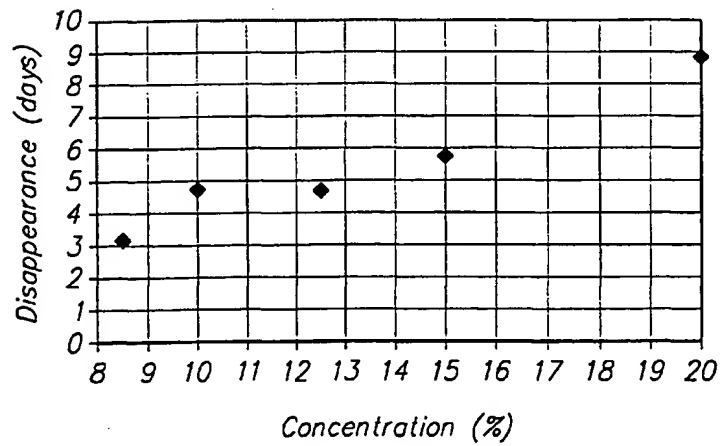


FIG. 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/28718

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONEElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,938,763 A (DUNN et al) 04 July 1995, abstract, cols. 1-20.	1-33
A	US 5,104,909 A (GRASEL et al) 14 April 1992, abstract, cols. 1-10.	1-33
A	US 5,426,148 A (TUCKER) 20 June 1995, abstract, cols. 1-22.	1-33
A	US 5,514,379 A (WEISSLEDER et al) 07 May 1996, abstract, cols. 1-16.	1-33
A	US 5,527,856 A (RHEE et al) 18 June 1996, abstract, cols. 1-24.	1-33
A	US 5,296,518 A (GRASEL et al) 22 March 1994, abstract, cols. 1-18.	1-33

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

20 MARCH 2000

Date of mailing of the international search report

04 APR 2000

 Name and mailing address of the ISA/US
 Commissioner of Patents and Trademarks
 Box PCT
 Washington, D.C. 20231
 Facsimile No. (703) 305-3230

 Authorized officer *Patricia Hightower*
 PATRICIA HIGHTOWER
 Telephone No. (703) 308-0661

INTERNATIONAL SEARCH REP RT

International application No.
PCT/US99/28718

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

A61F 2/00;
A61K 9/14, 9/50;
C08F 8/00, 283/04;
C08G 63/02, 63/08, 63/44, 63/48, 63/91, 69/10, 69/44, 69/48;
C08L 67/00, 71/02, 77/00;

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/ 423, 426, 484, 486, 488, 499;
523/ 113, 206;
524/ 592, 602, 612;
525/ 54.1, 55, 425, 937;
528/ 272, 288, 328, 354, 363;

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

424/ 423, 426, 484, 486, 488, 499;
523/ 113, 206;
524/ 592, 602, 612;
525/ 54.1, 55, 425, 937;
528/ 272, 288, 328, 354, 363;